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(57) Abstract

A polypeptide fragment or analog of CD26 capable of disrupting the naturally-occurring binding interaction between CD45 and CD26, and a method of screening such compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of: a) providing a first and a second sample of cells expressing both CD26 and CD45; b) incubating the first sample in the presence of a candidate compound; c) incubating the second sample in the absence of the candidate compound; d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody; e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

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HUMAN CD26 AND METHODS FOR USE

This application is a continuation-in-part of Morimoto et al., USSN 07/832,211. This invention was made at least in part with funds provided under grants from the United States Government (AI 12069, AR 33713). The Government has certain rights in the invention.

Background of the Invention

The field of the invention is human T cell activation antigens.

CD26 is a human T cell activation antigen originally identified by its reactivity with the monoclonal antibody Ta1 (Fox et al., *J. Immunol.* 133:1250, 1984). CD26 has recently been shown to be identical to human dipeptidyl peptidase IV (EC 3.4.14.5) (Ulmer et al., *Scand. J. Immunol.* 31:429, 1990; Barton et al., *J. Leukocyte Biol.* 48:291, 1990). Dipeptidyl peptidase IV (DPPIV) is a serine exopeptidase which is capable of cleaving x-proline or x-alanine (where x is any amino acid) from the amino terminus of certain peptides.

CD26 is recognized by a second monoclonal antibody, anti-1F7 (Morimoto et al., *J. Immunol.* 143:3430, 1989). Dang et al. (*J. Immunol.* 144:4092, 1990) report that solid phase-immobilized anti-1F7 mAb is capable of inducing proliferation of human $CD4^+$ T lymphocytes in conjunction with submitogenic doses of anti-CD3 or anti-CD2 antibodies. They suggest that the CD26 antigen is involved in CD3- and CD2-induced human $CD4^+$ T cell activation.

Summary of the Invention

In one aspect, the invention features a polypeptide fragment of CD26 lacking amino acid residues 3-9 of the latter sequence. (By "fragment" is meant a portion of CD26 that represents at least 50 consecutive residues of CD26. Such a fragment will preferably represent at least 100 residues of CD26, more preferably at least 200, and most preferably at least 500; it preferably includes the DPPIV active site residues at residues 627-631.) Such a fragment, in which the amino acid residues to the carboxy terminal side of residue 37 are preferably intact, is encoded by the nucleic acid sequence shown as CD26Δ3-9 (SEQ ID NO: 2). In preferred embodiments, the polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the product of signal peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

In another aspect, the invention features a polypeptide fragment of CD26 lacking residues 24-34 of the latter sequence. Such a fragment, in which the amino acid residues to the carboxy terminal side of residue 37 are preferably intact, is encoded by the nucleic acid sequence shown as CD26Δ24-34 (SEQ ID NO: 3). In preferred embodiments, the polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 3; the polypeptide is soluble under physiological conditions; and the polypeptide is substantially pure. Also within the invention is the product of signal peptidase proteolytic cleavage of this polypeptide, which would be a form of CD26 lacking residues 1-34, 1-35, 1-36, or 1-37.

In a related aspect, the invention features a plasmid encoding a polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2 (CDΔ3-9) or 3 (CDΔ24-34); this plasmid preferably includes an expression control sequence.

Polypeptide fragments of CD26 which are soluble under physiological conditions generally lack most or all of the hydrophobic amino acid residues found near the amino terminus of the polypeptide depicted in SEQ ID NO: 1. This can be accomplished by genetically manipulating a nucleic acid encoding CD26 to delete the hydrophobic residues, or to delete enough of the N-terminal amino acids (e.g., residues 3-9 or 24-34) to leave the resulting polypeptide susceptible to cleavage by signal peptidase. Other fragments of CD26 which are within the invention include those in which all or part of the putative dipeptidyl aminopeptidase catalytic site (Gly_{627} to Gly_{631}) is deleted. Such fragments, which include *inter alia* the deletion mutant shown in Fig. 15 (SEQ ID NO: 11); fragments having additional deletions such as those in Δ3-9 (SEQ ID NO: 2) and Δ24-34 (SEQ ID NO: 3); and those missing the entire signal peptide region up to Ala_{35}, Thr_{36}, Ala_{37} or Asp_{38}, would constitute enzymatically inactive fragments of CD26 useful in the screening assays of the invention, as well as for inhibiting complex formation between CD26 and/or CD45 and p43. Along the same lines, a mutant form of CD26 (or a fragment thereof) which lacks DPPIV activity can be generated by replacing one of the residues in the active site with a different amino acid (e.g., by replacing Ser_{629} with Ala).

By "substantially pure" is meant a polypeptide or protein which has been separated from biological macromolecules, (e.g., other proteins, carbohydrates,

etc.) with which it naturally occurs. Typically, a protein or polypeptide of interest is substantially pure when less than 25% (preferably less than 15%) of the dry weight of the sample consists of such other macromolecules.

By "physiological conditions" is meant an aqueous solution, whether *in vivo* or *in vitro*, having a pH and salt concentration similar to that found in serum. Phosphate buffered saline is an example of a commonly used buffer in which a polypeptide that is soluble under physiological conditions would be soluble.

By "substantially identical to CD26" is meant that at least 80%, preferably at least 90%, more preferably at least 95%, most preferably at least 99%, of the amino acid sequence is identical to that of the corresponding portion of CD26, and any non-identical amino acids in the sequence are amino acid substitutions, preferably conservative, which do not eliminate the biological activity of the molecule.

By "plasmid" is meant an extrachromosomal DNA molecule which includes sequences that permit replication within a particular host cell.

By "expression control sequence" is meant a nucleotide sequence which includes recognition sequences for factors that control expression of a protein coding sequence to which it is operably linked. Accordingly, an expression control sequence generally includes sequences for controlling both transcription and translation: for example, promoters, ribosome binding sites, repressor binding sites, and activator binding sites.

In another aspect, the invention features a polypeptide fragment of CD26 capable of disrupting the naturally-occurring binding interaction between CD45 and CD26. Polypeptides which disrupt the interaction between

CD26 and CD45 can be identified, for example, using the immunoprecipitation assay described below.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

(c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody;

(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

As used herein, an anti-CD26 antibody is one capable of forming a specific immune complex with CD26, i.e., the antibody binds directly to CD26 but does not substantially bind directly to other molecules in the assay of the invention.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

(c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD26 present in the first immunoprecipitate is less than the amount of CD26 present in the second immunoprecipitate, the presence of a lesser amount of CD26 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

In another aspect, the invention features a monoclonal antibody which, when contacted under physiological conditions with a cell (preferably a eukaryotic cell such as a mammalian cell) expressing CD26 and CD45, interferes with the association of CD26 and CD45; and a method for assaying for such an antibody.

In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD45 on its surface; and

(b) introducing into the cell a nucleic acid encoding CD26, such that the cell expresses CD26 on its surface.

In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD26 on its surface; and

(b) introducing into the cell a nucleic acid encoding CD45, such that the cell expresses CD45 on its surface.

In other aspects, the invention includes a cell transfected with a nucleic acid encoding CD26, the cell expressing both CD26 and CD45 on its surface; and a cell transfected with a nucleic acid encoding CD45, the cell expressing both CD26 and CD45 on its surface. In preferred embodiments, the cells are T-cells such as Jurkat cells.

In another aspect, the invention features a method which includes:

(a) providing a cell which expresses neither CD26 nor CD45 on its surface; and

(b) transfecting the cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

In yet another aspect, the invention includes a method of generating a hybridoma cell, which method includes:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

(b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line (i.e., a line of cells which can be maintained indefinitely in culture) to produce a hybridoma cell.

In a related aspect, the invention features a hybridoma cell generated by:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

(b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line to produce a hybridoma cell, wherein the hybridoma cell produces a monoclonal antibody specific for CD26. Applicable methods of inducing an immune response in an animal by using cells as the antigen, and fusing B lymphocytes with immortal cells to produce hybridoma cells, are well known to those of ordinary skill in the art of making hybridomas. The resulting hybridomas are then cloned and screened for production of monoclonal antibodies which bind to cells expressing the CD26 antigen, but not to identical cells which do not express the CD26 antigen.

Also within the invention are cell-free preparations of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof. Such complexes may be conveniently prepared by recombinant expression of each of the relevant polypeptides in a manner that prevents their being anchored to the cellular membrane (e.g., by use of a soluble fragment of each), or by isolation of the full-length proteins from a cell membrane preparation, and by combining the two polypeptides to form the desired complex either before or after removal of contaminating cellular constituents. Such complexes would be useful, e.g., for generating monoclonal antibodies specific for the complex, and for screening for compounds capable of interfering with the association of CD26 and CD45.

Also within the invention are purified preparations of p43, a 43 kDa molecule which, like CD45, associates with CD26 in cells and therefore is thought to play a role in T cell activation, and cell-free preparations of CD26 (or a fragment thereof) complexed with p43 (or a fragment thereof). The screening assay

described above for compounds capable of inhibiting the interaction of CD26 and CD45 can be readily adapted to detect compounds (including fragments of CD26 or p43) capable of inhibiting the interaction of CD26 and p43.

Also within the invention is a therapeutic composition containing a fragment of CD26 (e.g., water-soluble CD26), in a pharmaceutically acceptable carrier (e.g., saline or any aqueous or nonaqueous substance which is suitable for injection), or intact CD26 incorporated into a liposomal preparation or other carrier substance suitable for a polypeptide such as CD26. Such a therapeutic composition can be used in a method for modulating the immune response of a patient (e.g., enhancing the immune response of an immunosuppressed patient) by administering the composition by any appropriate means to the patient. It is expected to be particularly useful for the treatment of immunosuppression in a patient infected with human immunodeficiency virus (HIV) and having AIDS or AIDS-related complex, but may also be used where the patient's immune system is depressed as a result of treatment with an immunosuppressive compound, or acquired immunodeficiency of undetermined etiology, or congenital immunodeficiency.

The compounds of the invention are, when combined with a pharmaceutically acceptable carrier, also useful as vaccine adjuvants, to be administered to an individual vaccinee in conjunction with (i.e., immediately before, after, or along with) a vaccine antigen in order to enhance the immune response produced by such antigen. Examples of vaccine antigens which may be used with the adjuvant of the invention include those containing chemically inactivated or genetically engineered viral or bacterial products, such as diphtheria or pertussin

toxoid or recombinant viral proteins, and those containing live but attenuated virus or bacteria.

The assays described herein may be used to screen candidate immunosuppressive compounds by a method including the steps of (a) contacting a lymphocyte with CD26 or a fragment of CD26 in the presence of a candidate compound, and (b) determining whether the candidate compound inhibits the CD26-induced proliferation of the lymphocyte, such inhibition being an indication that the candidate compound has immunosuppressive activity. The assays may instead be used to screen CD26 fragments for immunostimulatory activity. One such assay would include the following steps: (a) contacting a lymphocyte with a candidate CD26 fragment, and (b) determining whether the fragment increases the rate of proliferation of the lymphocyte, such increase being an indication that the fragment has immunostimulatory activity. Alternatively, one could simply assay the fragment for dipeptidyl peptidase IV activity, such activity being an indication that the fragment has immunostimulatory activity.

Also within the invention is a solid matrix material (e.g. Affi-Gel™ (Bio-rad)) to which CD26 or a fragment thereof is attached.

CD26 is known to play a role in T cell activation. By interfering with the normal functioning of CD26, one can control the process of T cell activation, and thus prevent such unwanted immune responses as transplant rejection and certain autoimmune diseases. The information disclosed herein concerning proteins with which CD26 associates on the T cell provides the means for designing and screening compounds that interfere with CD26 function in the cell.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Detailed Description

The drawings are first briefly described.

Drawings

Fig. 1 depicts the nucletide sequence and deduced amino acid sequence (SEQ ID NO:1) of the cDNA clone for human CD26.

Fig. 2 depicts the results of an indirect fluoresence staining assay.

Fig. 3 is a pair of photographs of gels illustrating the results of immunoprecipitation analysis (panel A) and enzymatic activity analysis (panel B).

Fig. 4 is a set of graphs depicting the results of a $[Ca^{2+}]_i$ mobilization assay.

Fig. 5 is a graph illustrating the effect of various treatments on interleukin-2 production.

Fig. 6 is a photograph of a gel illustrating the results of immunoblotting analysis.

Fig. 7 depicts the results of FACS analysis.

Figs. 8-12 are photographs of gels illustrating the results of immunoprecipitation assays.

Fig. 13 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of Δ3-9 (SEQ ID NO: 2) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

Fig. 14 is a representation of the amino acid sequence of CD26 in which the deleted amino acids of Δ24-34 (SEQ ID NO: 3) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

Fig. 15 depicts the amino acid sequence of a CD26 fragment lacking a portion of the carboxy terminal region of CD26 (SEQ ID NO: 11).

Fig. 16 is a graph illustrating the effect of soluble CD26, soluble CD45, and soluble CD4 on PBL proliferation.

Sequencing and Characterization of CD26

Described below is the cloning and sequencing of a full-length CD26 cDNA. Also described are a series of experiments which demonstrate that: (1) modulation of CD26 from the surface of T lymphocytes leads to enhanced CD3ζ phosphorylation and increased CD4-associated $p56^{lck}$ tyrosine kinase activity; (2) CD26 is comodulated with CD45; and (3) CD26 and CD45 are closely associated.

Cells and Antibodies

Human peripheral blood mononuclear cells (PBMC), E rosette-positive cells and PHA-activated T cells for use in the experiments described below were prepared as follows. Human PBMC were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (LKB Biotechnology, Inc., Piscataway, NJ). Unfractionated mononuclear cells were separated into E rosette-positive (E+) and E rosette-negative (E-) populations, and the E+ cells were depleted of contaminating monocytes as described (Morimoto et al., *J. Immunol.* 134:3762, 1985; Morimoto et al., *J. Immunol.* 134:1508, 1985; Matsuyama et al., *J. Exp. Med.* 170:1133, 1989). These T cells were used for experiments involving T cells in this report. E+ cells were stimulated with PHA (0.25 μg/ml) and rIL-2 (40 U/ml) for 7 days in RPMI 1640 medium supplemented with 10% human AB serum, 4mM L-glutamine, 25 mM HEPES buffer, 0.5% sodium bicarbonate, and 1% penicillin/streptomycin (culture medium) and used as PHA blasts. The monoclonal antibodies used were anti-CD26 (Ta1/4EL-1C7, IgG_1; 1F7, IgG_1; 5F8, IgG_1), and anti-CD3 (T3/RW24B6; IgG_{2b}) (Fox et al., *J. Immunol.* 133:1250, 1984; Morimoto et al., *J. Immunol.* 143:3430, 1989; Morimoto et al., *J. Immunol.* 134:3762, 1985). Anti-CD29

(4B4; IgG_1) (Morimoto et al., *J. Immunol.* 134:3762, 1985) was used as an isotype-matched control throughout.

Isolation of a CD26 cDNA

To isolate a CD26 cDNA, a cDNA library was constructed from mRNA isolated from human PHA-activated T cells using the CDM7 vector. Briefly, poly(A)+ RNA was prepared from 4-day-old PHA-activated T cells by the guanidinium isothiocyanate method (Chirgwin et al., *Biochem.* 18:5294, 1979), and an expression library was prepared as previously described, except that the vector CDM7, a precursor to CDM8 lacking polyoma sequences, was employed (Aruffo et al., *Proc. Natl. Acad. Sci. USA* 84:8573, 1987; Seral et al., *Proc. Natl. Acad. Sci. USA* 87:3365, 1987). Recombinant hybrid plasmids were transfected into COS cells, and CD26 expressing cells were immunoselected with the monoclonal antibody, anti-Ta1 (Aruffo et al., *supra*; Seed et al., *supra*). Reactive cells were retained on antibody coated dishes, and plasmids were recovered from transfected cells. Plasmid DNAs were further selected by three additional rounds of transfection and immunoselection. Two of eight clones thus isolated were found to encode anti-Ta1 reactive determinants. The two clones were identical by restriction enzyme fragment mapping.

Sequencing of both strands of the isolated 2.9 kb CD26 cDNA by the dideoxy sequencing method revealed a 2298 base pair open reading frame beginning with an ATG at nucleotide 11 which conforms to consensus translational initiation sites (Fig. 1). The deduced CD26 structure is a 766 amino acid residue polypeptide with a molecular weight of approximately 88,300 (SEQ ID NO: 1).

Predicted Structure of CD26

The predicted CD26 polypeptide has a single stretch of hydrophobic amino acids in the N-terminal region between residues 7 and 28 (Fig. 1, boxed), which is sufficiently long and hydrophobic to span a lipid bilayer (Davis et al., *Cell* 41:607, 1985). The sequence is preceded by six N-terminal residues which contain polar and charged residues, and is followed by charged residues that would not allow cleavage by signal peptidase (von Heijne, *Nucl. Acids Res.* 14:4683, 1986). This sequence thus has the characteristics of a signal sequence of a type II membrane protein, which serves both to direct the translocation of the nascent protein across the membrane of the rough endoplasmic reticulum, and to anchor the mature protein in the membrane (Hong et al., *supra*, 1990; Shipp et al., *Proc. Natl. Acad. Sci. USA* 85:4819, 1988; Thomas et al., *J. Clin. Invest.* 83:1299, 1989). Furthermore, the fact that potential N-glycosylation sites are located in the carboxy side of the hydrophobic core (Fig. 1, short underlines) suggests that CD26 is a type II membrane protein. Therefore, the N-terminal 6 amino acid residues are predicted to be cytoplasmic, and the next 22 amino acids, which are primarily hydrophobic, are predicted to transverse the cytoplasmic membrane. The 738 C-terminal amino acids constitute the predicted extracellular domain of CD26.

The predicted extracellular domain of CD26 may be conveniently divided into three regions: an N-terminal glycosylated region (residues 29 to 323), a relatively cysteine-rich middle section (residues 324 to 551), and a C-terminal region (residues 552 to 766) (Fig. 1). The N-terminal region contains 8 of the 10 potential attachment sites for N-linked glycans (Fig. 1, short underlines) (Marshall, *Ann. Rev. Biochem.* 41:673, 1972), and one of the 12 cysteine residues (Fig. 1, asterisks). In

contrast, the subsequent cysteine-rich section contains 9 cysteines but only one N-linked glycosylation site. The C-terminal region contains two cysteines, one N-linked glycosylation site and a potential catalytic site (Fig. 1, double underline), the sequence G-W-S-Y-G at position 627 to 631. This sequence fits the consensus G-X-S-X-G found in the active sites of serine proteases and esterases, although tryptophan and tyrosine flanking the catalytic serine are unusual residues at these positions (Brenner, *Nature* 334:528, 1988).

Homology with the Other Proteins.

The predicted amino acid sequence of the human CD26 antigen (SEQ ID NO: 1) is 85% homologous to the deduced rat DPPIV enzyme sequence predicted from cDNAs isolated from rat liver and kidney libraries. Considering this high degree of homology and the fact that anti-Ta1 antibody reacts with human liver and kidney epithelium (Mobius et al., *Exp. Immunol.* 74:431, 1988), the DPPIV enzyme present in those tissues is probably the functional counterpart of the CD26 antigen. This high degree of homology also supports the prediction of the membrane topology of CD26, because rat DPPIV has been shown to be a type II membrane protein (Hong et al., *supra* 1990).

Aside from the signal sequence, the greatest homology between rat (Ogata et al., *supra*) and human CD26/DPPIV proteins is in the C-terminal region, which includes the putative catalytic site. In fact, the sequences are identical from residues 624 to 724, and 94% homologous from residues 552 to 766. This C-terminal region is 46% homologous to a region of the predicted yeast aminopeptidase B (DPAPB) sequence (Roberts et al., *J. Cell. Biol.* 108:1363, 1989). Further, CD26 amino acid residues 107 to 233 are 36% homologous to DPAPB. The yeast DPAPB enzyme is also a type II membrane dipeptidyl

aminopeptidase, and is involved in the maturation of the yeast pheromone alpha factor. The putative catalytic sequence G-W-S-Y-G is conserved between human and rat CD26/DPPIV and yeast DPAPB.

Recently the structures for CD10 and CD13 were determined by cDNA cloning (Shipp et al., *supra*, Thomas et al., *supra*). These antigens are ectoenzymes which have neutral endopeptidase [EC. 3.4.24.11] and aminopeptidase N [EC. 3.4.11.2] activities, respectively. Although CD10 and CD13 are also type II membrane proteins, there is no significant sequence homology between these enzymes and CD26.

Although the CD26 antigen is known to be a functional collagen receptor (Dang et al., *J. Exp. Med.* 172:649, 1990), a homology search did not find significant homology with any other known collagen-binding proteins such as fibronectin, CD11b and the integrins.

Characterization of CD26 Antigen expressed on Transfected Jurkat Cells

To characterize the cDNA-encoded CD26 antigen, the human T cell leukemia line, Jurkat, was transfected with the expression plasmid pSRα26, in which the CD26 cDNA was placed under the control of the SRα promoter. Briefly, the CD26 cDNA insert was cloned into the *Pst*I and *Eco*RI sites of the plasmid pCDLSRα296 (Takebe et al., *Mol. Cell. Biol.* 8:466, 1988) by blunt-end ligation to create the CD26 expression plasmid, pSRα-26. pSRα-26, digested with *Sal*I, and pSV2neo-SP (confers neomycin resistance to host cells; Streuli et al., *EMBO J.* 8:787, 1989), digested with *Pvu*I, were used to co-transfect Jurkat cells according to Streuli et al. (*supra*). Transfectants were initially selected in RPMI1640 supplemented with 10% fetal calf serum, 4mM glutamine and 1.0 mg/ml Geneticin (Gibco/BRL, Bethesda, MD). Subsequently, the

concentration of Geneticin was gradually decreased to 0.25 mg/ml during the selection period. Geneticin-resistant clones were further screened for CD3 and CD26 antigen expression by cell-surface staining as described below. Transfectants were maintained in the above medium containing 0.25 mg/ml Geneticin.

Staining of cell surface antigens with monoclonal antibodies and flow cytometry analyses using an EPICS V cell sorter (Coulter) were performed as described by Dang et al. (*J. Immunol.* 144:4092, 1990).

Parental Jurkat cells do not express detectable amounts of the CD26 antigen as determined by cell surface staining (Fig. 2), or by a binding assay with radiolabeled Ta1 monoclonal antibody. Northern blotting analysis revealed that this cell line also does not express CD26 mRNA even after phorbol 12-myristate 13-acetate (PMA) treatment, which is known to induce CD26 expression (Dang et al., *J. Immunol.* 145:3963, 1990). Referring to Fig. 2, the Jurkat-CD26 transfectant 26.C28 had high expression of the CD26 antigen. On the other hand, another Jurkat-CD26 clone, 26.24, expressed only moderate levels of the antigen. Both transfectants were reactive with three anti-CD26 monoclonal antibodies (Ta1, 1F7, and 5F8) which define three distinct CD26 antigen epitopes.

To study whether the CD26 antigen expressed on Jurkat T cell lines had the same characteristics as that on peripheral blood lymphocytes, immunoprecipitation experiments were carried out.

Briefly, cell surface proteins were labelled with lactoperoxidase-catalyzed iodination as described by Morimoto et al., (*J. Imunnol.* 143:3430, 1989). Immunoprecipitations (employing an NP-40 lysis buffer) using 1F7 monoclonal antibody were performed as described by Morimoto et al. (*supra*, 1989). Immunoprecipitated

proteins were separated by 8% SDS-PAGE under reducing conditions.

Referring to Fig. 3 (panel A), 1F7 monoclonal antibody immunoprecipitated a 110 kDa protein from the CD26 transfected Jurkat cells (lanes 2 and 3) as well as from PHA blasts (lane 4). There was no detectable 110 kDa band in nontransfected (lane 1) and vector-only transfected Jurkat cells. Control anti-4B4 monoclonal antibody immunoprecipitated a comparable amount of 130 kDa protein from each of the cell lines. Interestingly, 1F7 immunoprecipitated an additional 43 kDa protein from both transfectants and PHA blasts. Similar results were observed using peripheral blood T cells. This 43 kDa protein may contribute to T cell activation through its association with CD26.

DPPIV enzymatic activity was measured using an Enzyme Overlay Membrane system (EOM, Enzyme System Products, Dublin, CA). Briefly, lysates were incubated with SDS sample buffer for 1 hr at room temperature and separated by SDS-PAGE under non-reducing conditions. Following electrophoresis, the EOM moistened with 0.5M Tris-HCl, pH 7.8, was placed on the surface of the gel and this sandwich was incubated for 20 min in a humidified box at 37°C. The reaction was monitored by long wavelength ultraviolet light. Referring to Fig. 3, panel B, DPPIV enzymatic activity was associated with a 160 kDa protein in both transfectants (lanes 2 and 3) and PHA blasts (lane 4), but not in parental Jurkat cells (lane 1), or vector-only transfected cells. It should be noted that the DPPIV enzyme activity was stable in both non-reducing and reducing conditions but disappeared after boiling of the samples. While the apparent molecular weight of CD26 was 160,000 for preparations that were not boiled prior to electrophoresis, the molecular weight of CD26 antigen was 110,000 if the

protein was boiled prior to SDS-PAGE analysis. Similar results have been reported for rat hepatocyte DPPIV (Walburg et al., *Exp. Cell. Res.* 158:509, 1985). Taken together, the above-described results indicate that the CD26 antigen expressed on the transfected Jurkat cells was the same as that on peripheral blood T cells.

Functional Analysis of CD26 Antigen on Jurkat Transfectants

To determine whether the CD26 antigen expressed on transfected Jurkat cells has biological activity similar to that of CD26 expressed on peripheral blood T cells, we examined $[Ca^{2+}]_i$ mobilization induced by CD26 antigen triggering.

Briefly, loading of indo-1 pentaacetoxymethyl ester (Calbiochem, San Diego, CA) into cells and the measurement of its fluorescence by flow cytometry were performed as described by (Blue et al., *J. Immunol.* 140:376, 1988). Indo-1-loaded cells were preincubated for 1-2 minutes with antibodies and the basal intracellular calcium levels were determined for 33 seconds before the addition of polyclonal goat anti-mouse antibody (10 μg/ml) (Tago, Burlingame, CA). The RW24B6 anti-CD3 antibody was titrated in this system to determine the submitogenic dose for triggering each cell type. After preincubation of each transfectant with anti-CD26 and/or a submitogenic dose of anti-CD3, anti-mouse antibody was added (time point of addition indicated by small arrows in Fig. 4). Antibody concentrations were 1 μg/ml for anti-1F7 and 20 ng/ml for anti-CD3.

Referring to Fig. 4, crosslinking of anti-CD26 and submitogenic doses of anti-CD3 with goat anti-mouse immunoglobulin on CD26 transfectants resulted in greater $[Ca^{2+}]_i$ mobilization than crosslinking of anti-CD3 alone. These antibodies did not induce $[Ca^{2+}]_i$ mobilization

without cross-linking. It is well known that the $[Ca^{2+}]_i$ mobilization signal is divided into two phases: the initial transient rise, and the sustained increase phase (Gardner, *Cell* 59:15, 1989; Goldsmith et al., *Science* 240:1029, 1988). For both CD26 transfectants, the anti-CD26 and anti-CD3 crosslinking induced a strong initial $[Ca^{2+}]_i$ increase (Fig. 4). In addition, for the clone 26.C28, crosslinking induced a sustained increase of the $[Ca^{2+}]_i$ level as well (Fig. 4). The differential pattern of $[Ca^{2+}]_i$ mobilization of the two transfectants may be attributed to the difference in the amount of CD26 antigen expressed by these two transfectants. The enhanced $[Ca^{2+}]_i$ mobilization was specific because, as was reported for peripheral blood T cells (Dang et al., *J. Immunol.* 145:3963, 1990), crosslinking of the CD26 antigen alone did not induce $[Ca^{2+}]_i$ mobilization. Furthermore, crosslinking of anti-CD26 and anti-CD3 did not enhance the $[Ca^{2+}]_i$ mobilization of nontransfected or vector-only transfected Jurkat cells, and crosslinking of the isotype-matched control antibody, anti-4B4, did not result in enhanced $[Ca^{2+}]_i$ mobilization of the transfectants. Similar to the data observed with transfectants, a small but significant transient rise in $[Ca^{2+}]_i$ mobilization was observed in normal resting T cells following CD26 and CD3 crosslinking.

IL-2 production by transfected cells cultured in antibody-coated plates was measured as described by Dang et al., *J. Immunol.* 144:4092, 1990), except that the cell concentration was adjusted to $2x10^6$ cell/ml. After 24 hr of culture, supernatants were assayed for IL-2 production using ELISA (R&D system, Minneapolis, MN). Referring to Fig. 5, incubation of the clone 26.C28 transfectants with solid-phase-immobilized anti-1F7 and anti-CD3, which mimicked the crosslinking by anti-mouse antibody, induced the production of a significant amount of IL-2 (striped

bar), as compared to the control, vector-only transfected, Jurkat cells (solid bar). These results indicate that the CD26 Jurkat transfectants were functionally similar to peripheral blood T cells. Moreover, the above data indicate that the stimulatory effect of anti-CD26 and anti-CD3 crosslinking in T cells was in part mediated by an enhancement of $[Ca^{2+}]_i$ mobilization. Since it is well known that the transient rise, as well as the sustained increase, in $[Ca^{2+}]_i$ is necessary for IL-2 production (Gardner, *supra*; Goldsmith, *supra*), the sustained increase of the $[Ca^{2+}]_i$ observed in clone 26.C28 may be the basis for enhanced IL-2 production seen with the transfectant following anti-CD26 and anti-CD3 stimulation. Thus, the data obtained using Jurkat CD26 transfectants provide direct evidence that the CD26 antigen plays an integral role in T cell activation.

Co-association of CD26 and CD45

The experiments described below demonstrate that modulation of CD26 on the surface of T lymphocytes by anti-CD26 monoclonal antibody leads to enhanced phosphorylation of CD3 and increased $p56^{lck}$ tyrosine kinase activity. Modulation experiments described below demonstrate that CD26 is co-modulated with CD45. Finally, immunoprecipitation assays described below demonstrate that CD26 and CD45 are closely associated. Taken together, the results indicate that an interaction between CD26 and CD45 increases $p56^{lck}$ tyrosine kinase activity, CD3 chain phosphorylation, and T lymphocyte activation.

Enhancement of CD3ζ Phosphorylation Following anti-CD26 (1F7) Treatment

To evaluate the effect of anti-CD26 antibodies on one of the earliest signaling events in T cell

activation, we investigated their role in the tyrosine phosphorylation of CD3ζ.

Immunoblotting analysis of tyrosine phosphorylation of CD3ζ was performed as described by Vivier et al. (*J. Immunol.* 146:206, 1990). Briefly, peripheral blood T cells ($10x10^6$ per sample) were incubated in culture media alone or with anti-CD26 (1F7; 1:100 ascites dilution) for various times at 37°C. Cells were then extensively washed in ice cold PBS containing 5mM EDTA, 10mM NaF, 10mM sodium pyrophosphate, and 0.4mM sodium vanadate, then solubilized in lysis buffer (1% NP-40, 150mM NaCl, 50mM Tris HCl, pH 8.0, 5mM EDTA, 1mM PMSF, 10mM iodoacetamide, 10mM NaF, 10mM sodium pyrophosphate, 0.4mM sodium vanadate) for 15 min on ice. After removing insoluble material by centrifugation at 12,000 rpm for 15 min, samples were combined with an equal volume of sample buffer (2% SDS, 10% glycerol, 0.1M Tris [pH 6.8] 0.02% bromophenol blue), reduced with 5% 2-mercaptoethanol, and separated on 12% SDS-polyacrylamide gels. After separation on SDS-PAGE, cell lysates were transferred to nitrocellulose, and developed using ^{125}I-labelled anti-phosphotyrosine (UBI, NY; 100,000 cpm/ml in PBS containing 1% BSA). Affinity-purified anti-phosphotyrosine was iodinated to a specific radioactivity of 10-20 μCi/μg protein using iodobeads (Pierce Chemical Co., Rockford, IL).

Referring to Fig. 6, a 21 kD tyrosine phosphoprotein (p21), which has been previously identified in T cells stimulated with various stimuli as phosphorylated CD3ζ (Vivier et al., *supra*, 1990; Vivier et al., *J. Immunol.* 146:1142, 1991; Ashwell et al., *Annu. Rev. Immunol.* 8:139, 1990), was detected at a constitutive level in samples not treated with anti-CD26 (lane 1). Anti-CD26 treatment significantly increased the phosphorylation of CD3ζ over the constitutive level

after 1 hour of anti-CD26 incubation (lane 2). The level of phosphorylated CD3ζ gradually increased with time, reaching a maximum level after 4 hours of anti-CD26 incubation (lanes 3 and 4; 2 and 4 hours of anti-CD26 treatment respectively), and gradually decreased upon longer incubation (lanes 5 and 6; 6 and 8 hours of anti-CD26 treatment respectively). The total amount of CD3ζ chain (phosphorylated and non-phosphorylated) present, determined by immunoblotting the same membrane with an anti-CD3ζ mAb, was similar in all samples. Although anti-CD26 by itself can not induce T cell proliferation, these results show that CD26 modulation provides an initial T cell activation signal as measured by enhanced CD3ζ phosphorylation.

Comodulation of CD26 and CD45 by anti-CD26 Antibody (1F7) Treatment

The fact that the cytoplasmic domain of CD26 (DPPIV) in the rat includes only six amino acid residues suggests that CD26 might be associated with another molecule which acts in a signal transducing capacity, as has been found in the case of the IL-6 receptor and the IL-2 (p55) receptor (Taga et al., *Cell* 58:573, 1989; Robb et al.; *J. Exp. Med.* 165:1201; 1987). The experiments described below indicate that CD26 is associated with another cell surface molecule, CD45. Human peripheral blood T cells were used in the experiments described below and obtained as described by Dang et al. *J. Immunol.* 144:4092, 1990. Anti-CD26 (1F7) induced modulation was performed as previously described (by Dang et al. *J. Immunol.* 145:3963, 1990). Briefly, peripheral blood T cells were incubated overnight at 37°C in medium containing anti-CD26 (1F7) at 1:100 ascites dilution. Cells were then collected, washed and stained with anti-CD26 (1F7) and FITC-conjugated goat anti-mouse IgG; or they were stained with anti-CD45RA (2H4)-PE, anti-CD2-PE,

anti-CD3-PE (Coulter) or biotinylated anti-CD45RO (UCHL-1) and PE-conjugated avidin.

Flow cytometry analysis was performed using an Epics V cell sorter (Coulter Electronics) as previously described (Morimoto et al., *J. Immunol.* 143:3430, 1989).

The negative control of each fluorescence was less than 5%. The FACS analysis presented in Fig. 7 are representative of three separate experiments. As shown in Fig. 7, overnight incubation with anti-CD26 led to a significant reduction in CD26 expression on T cells. Interestingly, while CD26 modulation did not have any detectable effect on CD2, CD3 or CD45RA expression, the expression of CD45RO, particularly the high fluorescence peak of CD45RO, was markedly reduced. In addition, modulation of CD2, CD3, or CD4 with respective antibodies had no effect on CD45RO expression. Thus, the co-modulation of CD45RO induced by anti-CD26 treatment appears to be specific for this structure.

Co-immunoprecipitation of CD26 with CD45

The immunoprecipitation experiments described below provide evidence of a direct association between CD26 and CD45. Peripheral blood T cells ($50x10^6$) were labeled at the surface by lactoperoxidase-catalyzed iodination and immunoprecipitated from NP-40 lysis buffer (0.5% NP-40, 140mM NaCl, 1mM PMSF, 5mM EDTA, 50mM Tris HCl [pH 7.4]) or digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 150mM NaCl, 1mM PMSF, 20mM Triethanolamine [pH 7.8]) using anti-CD26 (Ta1, Coulter Immunology, Hialeah, FL; or 1F7, Dr. C. Morimoto, Dana-Farber Cancer Institute, Boston, MA) and anti-CD45 (GAP 8.3, Berger et al., *Human Immunol.* 3:231, 1981) as previously described by Morimoto et al. (*J. Immunol.* 143:3430, 1989) and Anderson et al. (*Nature* 341:159, 1989). All samples were analyzed under reducing conditions.

For immunodepletion studies, peripheral blood T cells were labeled and lysed in digitonin lysis buffer as described above. The lysates were precleared by four successive immunoprecipitations with anti-CD45 (GAP 8.3, American Type Culture Collection, Bethesda, MD) or anti-CD1 (T6) and then precipitated by anti-CD26 and anti-CD45.

Digestion with V8 protease from *S. aureus* was carried out during gel electrophoresis as described by Cleveland et al. (*J. Biol. Chem.* 252:1102, 1977). After the first gel electrophoresis, gel slices containing the high molecular weight proteins co-precipitated with CD26 and CD45 proteins were excised and polymerized into the stacking gel of a 15% SDS-polyacrylamide gel. 2.5 μg of V8 protease in 10 μl of sample buffer (0.1% SDS, 0.125M Tris-HCl [pH 6.8], 10% glycerol, 0.1% bromophenol blue) were added to wells above the polymerized gel slices. Gel electrophoresis was carried out uninterrupted for 12 hours.

Fig. 8 presents the results of immunoprecipitation analysis without prior depletion. Surface labeled T-lymphocytes were solubilized in NP-40 (lanes 1-4) or digitonin (lanes 5-8) and immunoprecipitated with anti-CD1 (T6) as a negative control (lanes 1 and 5); anti-CD26 (1F7, lanes 2 and 6); anti-CD26 (Ta1, lanes 3 and 7); or anti-CD45 (GAP 8.3, lanes 4 and 8).

While anti-CD26 (Ta1 and 1F7) antibodies precipitated a 110KD molecule from NP-40 lysates under reducing conditions, in digitonin lysates these same antibodies precipitated two major proteins at 180 and 190kD and minor bands at 205 and 220kD in addition to the 110KD band. These additional bands display similar mobility to the CD45 control immunoprecipitates. In this regard, utilizing digitonin lysates or chemical cross-linkers, others have found an association of CD45 with

Thy-1, CD3, and CD2 (Volarevic et al., *Proc. Natl. Acad. Sci. USA* 87:7085, 1990; Schraven et al., *Nature* 345:71, 1990).

To provide further evidence that the high molecular weight structure which co-precipitated with CD26 is CD45, we carried out both sequential immunodepletion and one-dimensional peptide mapping studies using V8 protease.

Fig. 9 presents the results of immunoprecipitation analysis of samples previously depleted for CD45 using anti-CD45 antibody (GAP 8.3, lanes 4-6) or, as a control, CD-1 using anti-CD1 antibody (T6, lanes 1-3). After depletion, anti-CD26 (1F7, lanes 1 and 4), anti-CD26 (Ta1, lanes 2 and 5), or anti-CD45 (GAP 8.3, lanes 3 and 6) was used for immunoprecipitation. As can be seen in Fig. 9, depletion of CD45 resulted in a complete loss of the high molecular weight structures in the CD26 immunoprecipitate (lanes 4, 5). Furthermore, V8 protease-dependent digestion of the high molecular weight molecules co-precipitated with either CD26 and CD45 yielded identical peptide patterns (Fig. 10). Although CD26 comodulated only with CD45RO (the 180kD isoform), the immunoprecipitation experiments suggest that CD26 is also associated with the 190kD isoform of CD45, and to a lesser degree, with the 205 and 220kD isoforms as well. These observations are consistent with earlier studies demonstrating that CD26 was preferentially expressed on CD45RO+ helper T cells, which are known to preferentially express both the 180 and 190kD isoforms of CD45 (Morimoto et al., *J. Immunol.* 143:3430, 1989; Rudd et al., *J. Exp. Med.* 166:1758, 1987; Terry et al., *Immunology* 64:331, 1988).

Enhancement of the Kinase Activity of $p56^{lck}$ following anti-CD26 (1F7) Treatment

Recent studies have demonstrated that the cytoplasmic domain of CD45 has PTPase activity which regulates T cell activation pathways through dephosphorylation of phosphotyrosine (Charboneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182, 1988; Ledbetter et al., *Proc. Natl. Acad. Sci., USA* 85:8628; Pingel et al., *Cell* 58:1055, 1989; Koretzky et al., *Nature* 346:66, 1990). One of the potential substrates for the CD45 PTPase is the tyrosine kinase $p56^{lck}$ (Osergaard et al., *Proc. Natl. Acad. Sci. USA* 86:8959, 1989; Mustelin et al., *Proc. Natl. Acad. Sci. USA* 86:6302, 1989), which itself may be involved in the CD3 chain phosphorylation (Veillette et al., *Nature* 338:257, 1989). CD26 may function in this system by enhancing CD3 phosphorylation through its association with CD45. If this model is correct, incubation with anti-CD26 (1F7) should alter $p56^{lck}$ kinase activity as measured by *in vitro* autophosphorylation.

To analyze *in vitro* kinase activity, samples of 3.0×10^7 T lymphocytes were incubated in culture media with anti-CD26 (1F7) for various periods of time at 37°C. Immunoprecipitation and kinase analysis was then carried out as described by Rudal et al. (*Proc. Natl. Acad. Sci. USA* 85:5190, 1988). Cells were then solubilized in lysis buffer (1% NP-40, 20 mM TRIS-HCl [pH 8.0], 150 mM NaCl, 0.4 mM sodium vanadate, 0.5 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate, 1 mM PMSF) for 30 min at 4°C. CD4 was immunoprecipitated from lysates containing equivalent amounts of total protein (500 μg) by a combination of anti-CD4 (19thy5D7; IgG2) and protein A-Sepharose. The immunoprecipitates were then washed extensively with lysis buffer prior to incubation with 30 μl of 25 mM Hepes containing 0.1% NP-40, and 10μCi of [λ-^{32}P]ATP (ICN,

Costa Mesa, CA). After incubation of 15-30 min at 25°C, the reaction was stopped by the addition of sample buffer and the reaction products were resolved on 9% SDS-PAGE.

As shown in Fig. 11, the PTK activity of $p56^{lck}$ precipitated with CD4 significantly increased after 1 hour of incubation with anti-CD26 (lane 2) compared to a no-anti-CD26 control (lane 1). The kinase activity was even higher after 2, 3 or 4 hours of incubation with anti-CD26 (lanes 3-6, respectively). Concomitantly, the expression of CD26 on T cells treated with anti-CD26 (1F7) began to decrease within 1 hour of incubation and continued to decline as previously described (Dang et al., *J. Immunol.* 145:3936, 1990). Similar results were obtained when another anti-CD26 (Ta1) antibody was used. Nevertheless, incubation of cells with control anti-Class I MHC or anti-VLA 4 mAbs did not alter $p56^{lck}$ activity. The above results support the notion that the interaction of CD26 with CD45 enhances $p56^{lck}$ activity.

The kinetics of $p56^{lck}$ PTK activity (Fig. 11) and tyrosine phosphorylation of CD3 (Fig. 6) showed a similar pattern. This similarity supports the conclusion that tyrosine phosphorylation of CD3 induced by anti-CD26 is related to the PTK activity of $p56^{lck}$. In addition, the similar kinetics also showed that the increase in $p56^{lck}$ PTK activity quickly affects the phosphorylation of CD3, as reported previously (Veillett et al., *supra*). While the peak of the $p56^{lck}$ PTK activity or phosphorylation of CD3 induced by various stimuli is observed within minutes (Vivier et al., *supra*; Veillette et al., *supra*), the peak of either $p56^{lck}$ or CD3 phosphorylation induced by anti-CD26 treatment was observed after hours. In this regard, although the close relationship between CD45 PTPase activity and $p56^{lck}$ PTK activity has been reported (Ostergaarol et al., *supra*; Mustelin et al., *supra*; Veillette et al., *supra*), the regulation of PTPase

activity of CD45 has not yet been established. Therefore, it is possible that the change in PTPase activity or the interaction between CD45 PTPase and $p56^{lck}$ may require a relatively long time period following anti-CD26 treatment. It is also possible that the interaction between CD45 PTPase and $p56^{lck}$ is via an indirect rather than a direct mechanism.

CD26 is broadly distributed on non-hematopoietic cells. However, since the expression of CD45 is largely restricted to leukocytes, the association between CD26 and CD45 is probably found only on leukocytes. On the other hand, membrane-linked PTPases such as CD45 have been found on non-hematopoietic cells (Streuli et al., *J. Exp. Med.* 168:1553, 1988; Streuli et al., *Proc. Natl. Acad. Sci. USA* 86:8698, 1989; Lau et al. *Biochem J.* 257:23, 1989). Although the functional role of CD26 on nonhematopoietic cells is unclear, it is possible that CD26 is associated with the membrane-linked PTPase on nonhematopoietic cells.

In summary, we have demonstrated that anti-CD26-induced modulation resulted in enhanced CD3 phosphorylation and increased $p56^{lck}$ PTK activity. Both observations are consistent with the enhanced proliferative response of T cells following CD26 modulation. These observations further suggest that the physical association of CD26 with CD45 may be key for CD26-mediated T cell signaling pathways. CD26 is known to be the membrane-associated ectoenzyme DPPIV which can cleave N-terminal dipeptides from polypeptides with either L-proline or L-alanine at the penultimate position. Although the natural ligand for CD26/DPPIV has not yet been established, binding of the natural substrate to the DPPIV enzyme may lead to cleavage and alteration in the biologic activity of the ligand. In light of the close proximity of the CD26 and CD45

molecules, it is possible that CD26 modulates the enzymatic activity of the CD45 PTPase or perhaps affects the accessibility of critical substrates. This process would then enhance T cell activation via the CD3 or CD2 pathway and could amplify the immune response *in vivo*. It should also be noted that increased numbers of CD26+ T lymphocytes have been found in both inflamed tissues and peripheral blood of patients with multiple sclerosis, Graves' Disease and rheumatoid arthritis (Hafler et al., *N. Engl. J. Med.* 312:1405, 1985; Nakao et al., *J. Rheumatol.* 16:904, 1989; Eguchi et al., *J. Immunol.* 142:4233, 1989), suggesting that these CD26+ T cells may play an important role in chronic inflammation and in subsequent tissue damage.

Soluble CD26 Fragments

Soluble fragments of CD26 are useful for interfering with CD26 activity. The fact that CD26 is a type II membrane protein suggests certain strategies for designing soluble fragments. For type II membrane proteins, the signal sequence used to transfer the protein across a membrane also serves as an anchor to the membrane. The cleavage of the signal sequence after protein transfer which usually occurs for other secreted proteins does not occur in type II transmembrane proteins. Thus, soluble forms of CD26 can be prepared by making its signal/anchor sequence accessible to a cellular proteolytic cleavage system. To accomplish this, the putative signal sequence of CD26 was shortened, as described below, since the 23 amino acid CD26 signal sequence is longer than most natural occuring cleavable signal sequences (von Heijne et al., *J. Mol. Biol.* 184:99, 1985). This is expected to result in proteolytic cleavage of the expressed polypeptide at or near one of the residues Ala Thr Ala corresponding to positions 35-37 of wild type CD26, yielding a soluble fragment of CD26

having at its amino terminus Ala_{35}, Thr_{36}, Ala_{37} or Asp_{38} of wild type CD26.

A first soluble CD26 construct is created by deleting the codons corresponding to amino acids 3-9 of intact CD26 (shown as the boxed amino acids in Fig. 13). The amino terminal sequence of the expressed polypeptide is MKGLLG-- (SEQ ID NO: 4) rather than the original MKTPWKVLLGLLG-- (SEQ ID NO: 5), and the potential proteolytic cleavage sites are shown as arrows in Fig. 13. This deletion mutant is prepared by oligonucleotide directed mutagenesis (see below) using the following oligonucleotide:

5'-ACGCCGACGATGAAGGGACTGCTGGGTGCT-3' (SEQ ID NO: 6).

A second construct is generated by taking advantage of the following rules proposed for signal peptide cleavage: (1) the residue in position -1 must be small, i.e., either Ala, Ser, Gly, Thr, Cys, Gln; (2) the residue in position -3 must not be aromatic (Phe, His, Tyr, Trp), charged (Asp, Glu, Lys, Arg), or large and polar (Asn, Gln); and (3) Pro must not be present at positions -3 through -1 (von Heijne, *Nuc. Acids Res.* 14:4683, 1986). Following these rules, we have designed a CD26 cDNA construct lacking codons corresponding to amino acids 24 to 34 of wild type CD26 (illustrated as the boxed amino acids in Fig. 14). This deletion mutant encodes the amino acid sequence --IITVATADSR-- (SEQ ID NO: 7) instead of the original --IITVPVVLLNKGTDDATADSR-- (SEQ ID NO: 8), and the potential proteolytic cleavage sites are shown as arrows in Fig. 14. This mutant is prepared by oligonucleotide-directed mutagenesis (see below) using the following oligonucleotide: 5'-ACCATCATCACCGTGGCTACAGCTGACAGT-3' (SEQ ID NO: 9).

Site-directed mutagenesis is performed as follows. The 3.0 kb CD26 cDNA fragment generated by the *Xba*I treatment of the original plasmid CDM7-CD26 is inserted into the *Xba*I site of pTZ19u (Bio-rad). A recombinant plasmid which inserts the cDNA inverse to the *lacZ* gene on the plasmid is identified by restriction enzyme mapping and used for subsequent mutagenesis.

Using single-stranded DNA prepared from this plasmid as a template and the previously-described oligonucleotides as primers, oligonucleotide-directed mutagenesis is performed by the method of Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985), using a commercially available kit (BioRad, Richmond, CA).

To obtain high level expression of soluble CD26, a new expression vector is constructed. First the *Xba*l CD26 cDNA fragment of pTZ19u-CD26 and the *Hind*III-*Xba*I vector fragment of Rc/CMV (Invitrogene, San Diego, CA) are treated with Klenow enzyme and ligated. The resulting plasmid is screened by restriction enzyme mapping for the insertion of the CD26 cDNA fragment under the control of the CMV promoter. This construct leaves one *Xba*I site just in front of the CD26 cDNA. Then, the *Mlu*I-*Xba*I CMV promoter DNA fragment of this plasmid DNA is exchanged with the *Hind*III-*Xba*I SRα promoter DNA fragment of pSRα-26 to give a final expression vector RcSRα-26. Next, the above mutant CD26 cDNAs are transferred to this expression vector. The *Xba*I-*Dra*III DNA fragment derived from the mutant cDNAs which encoded the mutant part and the wild type 2.0 kb *Dra*III-*Hind*III DNA fragment are ligated with the *Xba*I-*Hind*III vector fragment of RcSRα-26. The expression plasmid which has the Δ3-9 or Δ24-34 mutant CD26 cDNA is identified by restriction enzyme mapping and DNA sequencing. The resultant plasmids RcSRα-26.Δ3-9 and RcSRα-26.Δ24-34 are used to transfect Jurkat cells or CHO cells.

Jurkat cells are transfected with these plasmids as described above except pSVneo-sp is omitted from the donor DNA mixture since the RcSRα plasmid already carries the neo resistance marker. Neo-resistant clones are screened by metabolic labelling and immunoprecipitation (Harlow et al., eds. *Antibodies: a laboratory manual*, Cold Spring Harbor Laboratory, 1988) for the expression of soluble CD26. The transfectants which produce a large amount of soluble CD26 are used for protein production.

CHO cells transfected with the DNA mixture of pMT2 and RcSRα-26.Δ3-9 or RcSRα-26.Δ24-34 are selected for their growing ability in α-medium and the production of soluble CD26. The expression of the soluble protein is amplified by culturing the transfected CHO cells in medium containing an increasing amount of MTX. Although both Jurkat cells and CHO cells can provide the soluble form of CD26, the protein produced by Jurkat cells is preferred because of its human T cell origin.

Another approach to making fragments of CD26 is illustrated by the following:

Ligation of the CD26 XbaI-SphI cDNA fragment to the vector RcSRα-26 XbaI-HindIII DNA fragment and the following synthetic DNA linker:

5′----------CATAGTAATCGATA

GTACGTATCATTAGCTATTCGA-------5′ (SEQ ID NO: 10)

introduces an in-frame stop codon that results in deletion of the segment of CD26 from amino acid 594 to the carboxy terminus of the wild-type protein. This deletion mutant, which is shown in Fig. 15 (SEQ ID NO: 11), lacks the putative catalytic site of CD26 and has a new carboxy terminus of --GDKIMHA (SEQ ID NO: 12).

CD26 Derivatives Capable of Disrupting CD26/CD45 Interaction

Other polypeptide fragments of CD26 can be produced by standard methods of protein synthetic chemistry, using the information disclosed herein to design appropriate polypeptides and assay them for biological activity. A preferred method of producing such fragments, however, is by the use of recombinant DNA techniques. For example, the sequence of CD26 given in Fig. 1 (SEQ ID NO:1) can be used to design oligonucleotides encoding fragments of CD26 containing deletions of nonessential CD26 amino acid residues from the beginning, the end, and/or any central portion of the protein; such oligonucleotides are chemically synthesized by known methods and inserted into expression vectors for expression of a polypeptide fragment of CD26. Alternatively, one may manipulate the CD26 coding regions of CD26 expression plasmids by site-directed mutagenesis, as disclosed above for two such fragments of CD26, or by insertion of a stop codon at an appropriate place in the coding sequence. The CD26 fragment can then be produced in transfected cultured cells in large quantities, purified by standard methods, and tested in an assay such as the immunoprecipitation assay described above, which is useful for identifying fragments capable of disrupting the interaction of CD26 and CD45. Briefly, surface-labeled peripheral blood T cells which express both CD26 and CD45 (or any mammalian cells transfected with cDNAs encoding CD26 and CD45 so that both proteins are functionally expressed on the cells' surfaces) are incubated in the presence and absence of a CD26 polypeptide fragment. The cells are lysed in digitonin lysis buffer, and anti-CD45 monoclonal antibody is used to immunoprecipitate CD45 and any proteins associated

with CD45. The amount of CD26 that co-precipitates with CD45 in the presence of a given polypeptide fragment can be determined by known methods (e.g., by densitometer readings of the labelled bands on an SDS-PAGE gel analyzing the constituents of an immunoprecipitate) and compared to the amount that co-precipitates with CD45 in the absence of the polypeptide fragment. Alternatively, one can instead use an anti-CD26 antibody and measure the relative amounts of CD45 that co-precipitate with CD26 in the presence and absence of the given polypeptide fragment. If an anti-CD26 antibody is used, it is preferred that the antibody does not substantially bind to the competitor CD26 polypeptide; such binding interferes with the assay. In either case, CD26 polypeptide fragments which interfere with the interaction between CD26 and CD45 will decrease co-precipitation.

An analysis similar to that described above can be used to identify polypeptide fragments of CD45 which disrupt CD26/CD45 interaction. When screening CD45 fragments, it is preferable to perform the immunoprecipitation with anti-0CD26 antibody.

Association of p43 with CD26

When CD26 is immunoprecipitated from surface-labelled T cells and the immunoprecipitate is analyzed on SDS-PAGE, two bands are typically seen: one at 110kDa, corresponding to CD26, and a second, much fainter band at 43kDa. This lower molecular weight protein is termed "p43". Fig. 12 illustrates one such experiment, in which E+ cells were labeled by lactoperoxidase-catalyzed iodination and lysed in NP-40 lysis buffer for immunoprecipitation as described above. Precipitates were analyzed by 9% SDS-PAGE. Lane 1: anti-CD1 (T6) as negative control; lane 2: anti-1F7; lane 3: anti-Ta1;

lane 4: anti-5F8 (another anti-CD26 monoclonal antibody); lane 5: anti-CD29 (4B4) as control. As shown in Fig. 12, anti-1F7 brought down an obvious 43kDa structure (lane 2) from surface-labeled T cells. On the other hand, this structure was detected faintly following anti-Ta1 or anti-5F8 precipitation (lanes 3 and 4). This band was not detected following anti-CD1 or anti-CD29 precipitation (lanes 1 and 5). Similar results were seen when the cells were human thymocytes or from the human T cell lines H9 or Peer IV (data not shown). In other anti-Ta1 or anti-5F8 immunoprecipitation experiments using T cells from other donors, the 43kDa band was sometimes more distinct than those shown in lanes 3 and 4 of Fig. 12. In addition, a third band at approximately 70 kDa is sometimes observed in these CD26 immunoprecipitation experiments. Because they are found in association with the 110 kDa CD26 molecule, both the 43 kDa molecule and the 70 kDa molecule may play important roles in T cell activation. Compounds (such as fragments of CD26) which interfere with the association of CD26 with either p43 or the 70 kDa molecule may be detected by means of a screening assay patterned on those described above with respect to CD26 and CD45.

It is thought to be unlikely that anti-1F7 cross-reacts with p43, since the density of the 43kDa band decreased after repeated preclearing by either anti-Ta1 or anti-5F8. Although the reasons for the variability in the detection of p43 are not clear, it is possible that the binding of anti-CD26 mAbs may generate conformational changes in CD26, affecting the association of the 43 kDa molecule with the 110 kDa molecule. It is also possible that the Ta1 or 5F8 epitope may be close to the association site between the 43 and 110 kDa molecules, such that binding of these mAbs may inhibit the association of these molecules with each other.

P43 may be purified by affinity chromatography, using an anti-CD26 monoclonal antibody to purify the CD26-p43 complex from T cell membranes. P43 may then be separated from CD26 by SDS-PAGE, followed by HPLC if further purification is necessary. Affinity chromatography with monoclonal antibodies, SDS-PAGE, and HPLC are all standard methods well known to those of ordinary skill in the art.

Hybridization probes based upon a partial amino acid sequence of the purified protein may be used to select p43 cDNA from a T cell library. Alternatively, the partial amino acid sequence can be used to design PCR primers for priming synthesis of a partial p43 cDNA on mRNA templates, using standard methods, and the resulting partial cDNA used as a probe to detect full-length p43 cDNA in a T cell library. This cDNA can be inserted in an expression plasmid and used to transfect cells which do not naturally express the p43 gene. Such cells may be used as an antigen to develop anti-p43 monoclonal antibodies, and also as a means to study the role of p43 in T cell activation. They can also be used in the screening assay referred to above.

Northern Analysis Using a CD26 cDNA Probe

Analysis of the degree of expression of CD26 in any given cell type or tissue type can be accomplished using the standard technique of Northern blotting, probing with a labelled, single stranded nucleic acid molecule derived from the coding region of CD26 cDNA. The probe would have a sequence based upon the sense strand of SEQ ID NO: 1, which is complementary to CD26 mRNA, and preferably would be at least 8 nucleotides in length (more preferably at least 14 nucleotides, and most preferably at least 30). The probe may contain most or all of the entire coding sequence of CD26 cDNA. Such an

assay, which would be useful for diagnosing conditions characterized by the over- or under-expression of CD26 in a given cell type, such as T cells, would include the following steps:

(a) providing a biological sample containing mRNA of a cell;

(b) contacting the sample with a single-stranded nucleic acid probe as described above; and

(c) detecting hybridization of the probe with the sample, which hybridization would be indicative of the presence of CD26 mRNA in the cell.

Purification of Soluble CD26

To produce soluble CD26, CHO cells stably expressing CD26Δ3-9 (CD26 deleted for amino acids 3-9) were cultured in serum-free medium (CHO-S-SFM; GIBCO/BRL) containing 0.5 μM methotrexate. The culture supernatant was collected and proteins were precipitated using 75% ammonium sulfate. The resulting pellet was solubilized in PBS, dialyzed against PBS, and loaded on a ConA-Sepharose column (Pharmacia, Piscataway, NJ) equilibrated with 2xPBS/0.02% sodium azide. The column was washed with the equilibration buffer, and protein was eluted with 2xPBS/0.2M methyl α-D-mannopyranoside/0.02% sodium azide. A DPPIV assay (described below) was used to identify the CD26-containing fractions, which were pooled and loaded directly on a BSA-conjugated Affigel™ 10 column (Bio-rad) equilibrated with PBS/0.02% sodium azide. The flowthrough fraction was collected and applied to a 1F7-conjugated Affigel™ 10 column equilibrated with PBS. The column was washed with PBS, and soluble CD26 was eluted with PBS/3M sodium thiocyanate. The fractions containing DPPIV activity were pooled and dialyzed against PBS. The resulting

soluble CD26 was more than 95% pure as judged by gel electrophoresis.

Soluble CD26 and Cell Activation

Soluble CD26 was shown to stimulate antigen-dependent proliferation of peripheral blood lymphocytes *in vitro*. Assays were performed in triplicate wells in round-bottom plates using 0.2 ml/well standard culture medium consisting of RPMI1640 supplemented with 10% human AB serum, 4 mM L-glutamine, 25 mM HEPES buffer (Microbiological Associates), 0.5% sodium bicarbonate, and 50 μg/ml of Gentamicin (GIBCO). The cell concentration was 1.2×10^5 cells/well. Tetanus toxoid (Connaught Lab, Inc.) dialyzed against PBS was added to some of the wells to make a final concentration of 0.2 or 0.1 L.T. unit/ml (1/40 or 1/80 dilution of the original solution, respectively); the toxoid serves as soluble antigen in this assay. Purified soluble CD26 antigen, soluble LCA (leucocyte common antigen; CD45), or soluble CD4 was added at a final concentration of 1 μg/ml or 25 μg/ml. After 7 days culture in a CO_2 incubator, the cells were pulsed with 1 μCi/well of ^{3}H-thymidine. After a 16 hr incubation, the cells were harvested and the ^{3}H-thymidine incorporation was measured using a scintillation counter.

As shown in Fig. 16, in the presence of tetanus toxoid (diluted 80-fold or 40-fold), soluble CD26 (prepared as described above) stimulated PBL proliferation in a dose-dependent manner. This stimulation was greater than that observed when soluble CD45 or soluble CD4 was used instead of soluble CD26.

This assay can be used to screen fragments of CD26 to identify molecules capable of stimulating antigen-dependent immune cell proliferation. In addition, it can be used to assay for compounds capable of inhibiting soluble CD26-stimulated proliferation of lymphocytes. By

substituting an anti-CD3 antibody such as OKT3 (Kung et al. U.S. Patent 4,658,019, 4,361,549, and 4,654,210) for the tetanus toxoid in this assay, the ability of fragments of CD26 to stimulate antigen-independent immune cell proliferation can be determined.

CD26 Mutant

Standard methods of site-directed mutagenesis were used to produce a point mutation ($Ser^{629} \rightarrow Ala$) within the putative catalytic site of DPPIV activity of CD26. The enzymatic activity of the resulting mutant CD26 (CD26-629A) was examined by transfecting Jurkat cells with plasmids expressing intact CD26, CD26-629A, or vector only. Transformed cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, 50 μg/ml Gentamicin, and 0.25 mg/ml Geneticin (GIBCO), then harvested, lysed, and separated into a membrane fraction and a cytosol fraction (by the method of Siekierka et al., J. Immunol. 143:1580-1583, 1989). DPPIV activity of each fraction was measured in accordance with Hanski et al. (Exp. Cell Res. 178:64-72, 1988). Membranes from cells transformed with the plasmid encoding CD26-629A had almost no DPPIV activity, while membranes containing wild-type CD26 had substantial DPPIV activity. In addition, some DPPIV activity was observed in the cytosolic fraction of cells expressing wild-type CD26, but not cells expressing CD26-629A.

Although CD26-629A apparently lacks DPPIV activity, cells expressing CD26-629A were recognized by three anti-CD26 antibodies (1F7, Ta1(4EL), and 5F8) which recognize wild-type CD26, indicating that the mutant protein is expressed in the transformed cells, and suggesting that the mutation does not have a substantial effect on protein conformation.

Jurkat cells expressing wild-type CD26 were stimulated with anti-CD3 antibody (OKT3) and either anti-CD26 antibody (1F7) or PMA produced substantially more IL-2 than cells expressing CD26-629A or cells transformed with vector only. This suggests that the DPPIV activity of CD26 is important for both CD26-dependent and CD26-independent activation. It was also observed that, after stimulation with anti-CD26 and anti-CD3 antibodies, cells expressing the mutant form of CD26 produced more IL-2 than control cells that did not express either mutant or wild-type CD26, suggesting that DPPIV activity is not the only facet of CD26 which contributes to stimulation of IL-2 production in this system.

Use

Soluble CD26 and variants thereof are generally useful as immune response-stimulating therapeutics. For example, the compounds of the invention can be used for treatment of disease conditions characterized by immunosuppression: e.g., AIDS or AIDS-related complex, other virally- or environmentally-induced conditions, and certain congenital immune deficiencies. The compounds may also be employed to increase immune function that has been impaired by the use of immunosuppressive drugs such as certain chemotherapeutic agents, and therefore are particularly useful when given in conjunction with such drugs. When given as an adjuvant in conjuntion with a vaccine antigen, the compounds of the invention will boost the immune response triggered by the vaccine and thus increase the vaccine's protective potency. This would be particularly beneficial where the vaccinee is incapable of generating an optimal immune response without the use of such an adjuvant, as is the case for newborns or for persons undergoing renal dialysis or transplantation, or where the vaccine antigen is one which is poorly immunogenic.

Generally, the compounds of the invention will be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, or intraperitoneally. Optimal formulation and dosage can be readily determined by one of ordinary skill in the art of pharmacology, taking into account such factors as the biological half-life of the compound and the degree of immunostimulation desired. It is expected that a typical dose for a severely immunocompromised patient will be approximately 0.01 to 100 μg/kg/day. When utilized as a vaccine adjuvant, a typical single dose of the compound of the invention would be 0.1 to 100 μg.

Instead of soluble forms of CD26, intact CD26 or a form of CD26 which retains the membrane-anchoring amino terminal portion of native CD26, as well as all critical portions of the remainder of the molecule, can be incorporated into red cell "ghosts" or liposomes, so that the protein is expressed on the surface of the ghosts or liposomes. This form of CD26 is then suspended in a pharmaceutically acceptable carrier and introduced into the patient as described above, so that it can interact with the patient's immunological cells *in vivo*. Alternatively, peripheral blood lymphocytes can be withdrawn from the patient and treated with a CD26 compound of the invention (whether in soluble or membrane-bound form, or attached to a solid support by standard methodologies) *ex vivo*, prior to introducing the newly-stimulated lymphocytes into the same or a different patient.

As discussed above, the assay for enhancement of lymphocyte proliferation with soluble CD26 can be utilized to screen for compounds which inhibit such enhancement, and which therefore could be used to

interfere with CD26-stimulated proliferation of lymphocytes *in vivo*. The information provided above concerning the location of the DPPIV active site of CD26 provides a starting place for the design of compounds which will bind to the active site and thus potentially inhibit the stimulatory activity of CD26. Such compounds can first be tested for their ability to bind to CD26 by passing each such compound over a CD26 affinity column; compounds which bind to the column can then be assayed for their ability to inhibit soluble CD26-enhanced proliferation of lymphocytes *in vitro*, as described above. Such inhibitory compounds would be useful for the treatment of conditions characterized by an unwanted immune response: for example, autoimmune diseases such as systemic lupus erythematosis and rheumatoid arthritis.

Other Embodiments

The invention also includes analogs of CD26 and of fragments of CD26. The term "analogs" refers to polypeptide fragments of CD26 having conservative and/or non-conservative substitutions for some of the amino acids of naturally-occurring CD26, having D-amino acids in place of some or all of the corresponding L-amino acids, or having non-peptide bonds in place of some of the peptide bonds of CD26. Techniques for producing such analogs are well known in the art, and can be readily accomplished by those of ordinary skill. Preferably at least 85%, more preferably at least 95%, and most preferably at least 99%, of the amino acids in the analog are identical to the corresponding ones in CD26. It is important that the substitutions do not eliminate the ability of the polypeptide fragment to interfere with the naturally occurring association between CD26 and CD45, or the ability of the compound to stimulate proliferation of lymphocytes. In some instances, the removal of peptide bonds from a polypeptide compound is a desirable goal

because the presence of such bonds may leave the compound susceptible to attack by proteolytic enzymes. Additionally, such peptide bonds may affect the biological availability of the resulting therapeutic molecules. The removal of peptide bonds is part of a process referred to as "depeptidization". Depeptidization entails such modifications as replacement of the peptide bond (-CONH-) between two given amino acids with a spatially similar group such as $-CH_2CH_2-$, $-CH_2-O-$, -CH=CH-or $-CH_2S-$, generally by incorporating a non-peptide mimetic of the dipeptide into the chemically synthesized analog of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Tanaka, Toshiaki

(ii) TITLE OF INVENTION: HUMAN CD26 AND METHODS FOR USE

(iii) NUMBER OF SEQUENCES: 16

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(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

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(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	2924
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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             1               5                  10

GCT GCT GCG CTT GTC ACC ATC ATC ACC GTG CCC GTG GTT CTG CTG AAC    97
Ala Ala Ala Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn
     15                  20                  25

AAA GGC ACA GAT GAT GCT ACA GCT GAC AGT CGC AAA ACT TAC ACT CTA   145
Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu
 30                  35                  40                  45

ACT GAT TAC TTA AAA AAT ACT TAT AGA CTG AAG TTA TAC TCC TTA AGA   193
Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg
                 50                  55                  60

TGG ATT TCA GAT CAT GAA TAT CTC TAC AAA CAA GAA AAT AAT ATC TTG   241
Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu
             65                  70                  75

GTA TTC AAT GCT GAA TAT GGA AAC AGC TCA GTT TTC TTG GAG AAC AGT   289
Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser
         80                  85                  90

ACA TTT GAT GAG TTT GGA CAT TCT ATC AAT GAT TAT TCA ATA TCT CCT   337
Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro
     95                  100                 105

GAT GGG CAG TTT ATT CTC TTA GAA TAC AAC TAC GTG AAG CAA TGG AGG   385
Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg
110                 115                 120                 125

CAT TCC TAC ACA GCT TCA TAT GAC ATT TAT GAT TTA AAT AAA AGG CAG   433
His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln
                130                 135                 140

CTG ATT ACA GAA GAG AGG ATT CCA AAC AAC ACA CAG TGG GTC ACA TGG   481
Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp
            145                 150                 155

TCA CCA GTG GGT CAT AAA TTG GCA TAT GTT TGG AAC AAT GAC ATT TAT   529
Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr
        160                 165                 170

GTT AAA ATT GAA CCA AAT TTA CCA AGT TAC AGA ATC ACA TGG ACG GGG   577
Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly
    175                 180                 185

AAA GAA GAT ATA ATA TAT AAT GGA ATA ACT GAC TGG GTT TAT GAA GAG   625
Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu
190                 195                 200                 205
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                210                 215                 220

TTT TTA GCA TAT GCC CAA TTT AAC GAC ACA GAA GTC CCA CTT ATT GAA   721
Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu
            225                 230                 235

TAC TCC TTC TAC TCT GAT GAG TCA CTG CAG TAC CCA AAG ACT GTA CGG   769
Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg
        240                 245                 250

GTT CCA TAT CCA AAG GCA GGA GCT GTG AAT CCA ACT GTA AAG TTC TTT   817
Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe
    255                 260                 265

GTT GTA AAT ACA GAC TCT CTC AGC TCA GTC ACC AAT GCA ACT TCC ATA   865
Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile
270                 275                 280                 285

CAA ATC ACT GCT CCT GCT TCT ATG TTG ATA GGG GAT CAC TAC TTG TGT   913
Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys
                290                 295                 300

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            305                 310                 315

AGG ATT CAG AAC TAT TCG GTC ATG GAT ATT TGT GAC TAT GAT GAA TCC  1009
Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser
        320                 325                 330

AGT GGA AGA TGG AAC TGC TTA GTG GCA CGG CAA CAC ATT GAA ATG AGT  1057
Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser
    335                 340                 345

ACT ACT GGC TGG GTT GGA AGA TTT AGG CCT TCA GAA CCT CAT TTT ACC  1105
Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr
350                 355                 360                 365

CTT GAT GGT AAT AGC TTC TAC AAG ATC ATC AGC AAT GAA GAA GGT TAC  1153
Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr
                370                 375                 380

AGA CAC ATT TGC TAT TTC CAA ATA GAT AAA AAA GAC TGC ACA TTT ATT  1201
Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile
            385                 390                 395

ACA AAA GGC ACC TGG GAA GTC ATC GGG ATA GAA GCT CTA ACC AGT GAT  1249
Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp
        400                 405                 410

TAT CTA TAC TAC ATT AGT AAT GAA TAT AAA GGA ATG CCA GGA GGA AGG  1297
Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg
    415                 420                 425

AAT CTT TAT AAA ATC CAA CTT AGT GAC TAT ACA AAA GTG ACA TGC CTC  1345
Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu
430                 435                 440                 445
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AGT TGT GAG CTG AAT CCG GAA AGG TGT CAG TAC TAT TCT GTG TCA TTC  1393
Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe
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AGT AAA GAG GCG AAG TAT TAT CAG CTG AGA TGT TCC GGT CCT GGT CTG  1441
Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu
            465                 470                 475

CCC CTC TAT ACT CTA CAC AGC AGC GTG AAT GAT AAA GGG CTG AGA GTC  1489
Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val
        480                 485                 490

CTG GAA GAC AAT TCA GCT TTG GAT AAA ATG CTG CAG AAT GTC CAG ATG  1537
Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met
    495                 500                 505

CCC TCC AAA AAA CTG GAC TTC ATT ATT TTG AAT GAA ACA AAA TTT TGG  1585
Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp
510                 515                 520                 525

TAT CAG ATG ATC TTG CCT CCT CAT TTT GAT AAA TCC AAG AAA TAT CCT  1633
Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro
                530                 535                 540

CTA CTA TTA GAT GTG TAT GCA GGC CCA TGT AGT CAA AAA GCA GAC ACT  1681
Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr
            545                 550                 555

GTC TTC AGA CTG AAC TGG GCC ACT TAC CTT GCA AGC ACA GAA AAC ATT  1729
Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile
        560                 565                 570

ATA GTA GCT AGC TTT GAT GGC AGA GGA AGT GGT TAC CAA GGA GAT AAG  1777
Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys
    575                 580                 585

ATC ATG CAT GCA ATC AAC AGA AGA CTG GGA ACA TTT GAA GTT GAA GAT  1825
Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp
590                 595                 600                 605

CAA ATT GAA GCA GCC AGA CAA TTT TCA AAA ATG GGA TTT GTG GAC AAC  1873
Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn
                610                 615                 620

AAA CGA ATT GCA ATT TGG GGC TGG TCA TAT GGA GGG TAC GTA ACC TCA  1921
Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser
            625                 630                 635

ATG GTC CTG GGA TCA GGA AGT GGC GTG TTC AAG TGT GGA ATA GCC GTG  1969
Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val
        640                 645                 650

GCG CCT GTA TCC CGG TGG GAG TAC TAT GAC TCA GTG TAC ACA GAA CGT  2017
Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg
    655                 660                 665

TAC ATG GGT CTC CCA ACT CCA GAA GAC AAC CTT GAC CAT TAC AGA AAT  2065
Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn
670                 675                 680                 685

TCA ACA GTC ATG AGC AGA GCT GAA AAT TTT AAA CAA GTT GAG TAC CTC  2113
Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu
                690                 695                 700
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CTT ATT CAT GGA ACA GCA GAT GAT AAC GTT CAC TTT CAG CAG TCA GCT  2161
Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala
            705                 710                 715

CAG ATC TCC AAA GCC CTG GTC GAT GTT GGA GTG GAT TTC CAG GCA ATG  2209
Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met
        720                 725                 730

TGG TAT ACT GAT GAA GAC CAT GGA ATA GCT AGC AGC ACA GCA CAC CAA  2257
Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln
    735                 740                 745

CAT ATA TAT ACC CAC ATG AGC CAC TTC ATA AAA CAA TGT TTC TCT TTA  2305
His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu
750                 755                 760                 765

CCT TAGCACCTCA AAATACCATG CCATTTAAAG CTTATTAAAA CTCATTTTTG       2358
Pro
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TTTTCATTAT CTCAAAACTG CACTGTCAAG ATGATGATGA TCTTTAAAAT ACACACTCAA  2418

ATCAAGAAAC TTAAGGTTAC CTTTGTTCCC AAATTTCATA CCTATCATCT TAAGTAGGGA  2478

CTTCTGTCTT CACAACAGAT TATTACCTTA CAGAAGTTTG AATTATCCGG TCGGGTTTTA  2538

TTGTTTAAAA TCATTTCTGC ATCAGCTGCT GAAACAACAA ATAGGAATTG TTTTTATGGA  2598

GGCTTTGCAT AGATTCCCTG AGCAGGATTT TAATCTTTTT CTAACTGGAC TGGTTCAAAT  2658

GTTGTTCTCT TCTTTAAAGG GATGGCAAGA TGTGGGCAGT GATGTCACTA GGGCAGGGAC  2718

AGGATAAGAG GGATTAGGGA GAGAAGATAG CAGGGCATGG CTGGGAACCC AAGTCCAAGC  2778

ATACCAACAC GACCAGGCTA CTGTCAGCTC CCCTCGGAGA AAACTGTGCA GTCTGCGTGT  2838

GAACAGCTCT TCTCCTTTAG AGCACAATGG ATCTCGAGGG ATCTTCCATA CCTACCAGTT  2898

CTGCGCCTCG AGGCCGCGAC TCTAGA                                       2924
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 759
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Lys Gly Leu Leu Gly Ala Ala Ala Leu Val Thr Ile Ile Thr Val
 1               5                  10                  15

Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser
            20                  25                  30

Arg Lys Thr Tyr Thr Leu Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu
        35                  40                  45

Lys Leu Tyr Ser Leu Arg Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys
    50                  55                  60
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Gln Glu Asn Asn Ile Leu Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser
65 70 75 80

Val Phe Leu Glu Asn Ser Thr Phe Asp Glu Phe Gly His Ser Ile Asn
85 90 95

Asp Tyr Ser Ile Ser Pro Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn
100 105 110

Tyr Val Lys Gln Trp Arg His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr
115 120 125

Asp Leu Asn Lys Arg Gln Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn
130 135 140

Thr Gln Trp Val Thr Trp Ser Pro Val Gly His Lys Leu Ala Tyr Val
145 150 155 160

Trp Asn Asn Asp Ile Tyr Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr
165 170 175

Arg Ile Thr Trp Thr Gly Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr
180 185 190

Asp Trp Val Tyr Glu Glu Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp
195 200 205

Trp Ser Pro Asn Gly Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr
210 215 220

Glu Val Pro Leu Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln
225 230 235 240

Tyr Pro Lys Thr Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val Asn
245 250 255

Pro Thr Val Lys Phe Phe Val Val Asn Thr Asp Ser Leu Ser Ser Val
260 265 270

Thr Asn Ala Thr Ser Ile Gln Ile Thr Ala Pro Ala Ser Met Leu Ile
275 280 285

Gly Asp His Tyr Leu Cys Asp Val Thr Trp Ala Thr Gln Glu Arg Ile
290 295 300

Ser Leu Gln Trp Leu Arg Arg Ile Gln Asn Tyr Ser Val Met Asp Ile
305 310 315 320

Cys Asp Tyr Asp Glu Ser Ser Gly Arg Trp Asn Cys Leu Val Ala Arg
325 330 335

Gln His Ile Glu Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg Pro
340 345 350

Ser Glu Pro His Phe Thr Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile
355 360 365

Ser Asn Glu Glu Gly Tyr Arg His Ile Cys Tyr Phe Gln Ile Asp Lys
370 375 380

Lys Asp Cys Thr Phe Ile Thr Lys Gly Thr Trp Glu Val Ile Gly Ile
385 390 395 400

Glu Ala Leu Thr Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys
405 410 415

Gly Met Pro Gly Gly Arg Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr
420 425 430

Thr Lys Val Thr Cys Leu Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln
435 440 445

Tyr Tyr Ser Val Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg
450 455 460

Cys Ser Gly Pro Gly Leu Pro Leu Tyr Thr Leu His Ser Ser Val Asn
465 470 475 480

Asp Lys Gly Leu Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Lys Met
485 490 495

Leu Gln Asn Val Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu
500 505 510

Asn Glu Thr Lys Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe Asp
515 520 525

Lys Ser Lys Lys Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys
530 535 540

Ser Gln Lys Ala Asp Thr Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu
545 550 555 560

Ala Ser Thr Glu Asn Ile Ile Val Ala Ser Phe Asp Gly Arg Gly Ser
565 570 575

Gly Tyr Gln Gly Asp Lys Ile Met His Ala Ile Asn Arg Arg Leu Gly
580 585 590

Thr Phe Glu Val Glu Asp Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys
595 600 605

Met Gly Phe Val Asp Asn Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr
610 615 620

Gly Gly Tyr Val Thr Ser Met Val Leu Gly Ser Gly Ser Gly Val Phe
625 630 635 640

Lys Cys Gly Ile Ala Val Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp
645 650 655

Ser Val Tyr Thr Glu Arg Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn
660 665 670

Leu Asp His Tyr Arg Asn Ser Thr Val Met Ser Arg Ala Glu Asn Phe
675 680 685

Lys Gln Val Glu Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val
690 695 700

His Phe Gln Gln Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val Gly
705 710 715 720

Val Asp Phe Gln Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile Ala
725 730 735

Ser Ser Thr Ala His Gln His Ile Tyr Thr His Met Ser His Phe Ile
740 745 750

Lys Gln Cys Phe Ser Leu Pro
755

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 755
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
1 5 10 15

Leu Val Thr Ile Ile Thr Val Ala Thr Ala Asp Ser Arg Lys Thr Tyr
20 25 30

Thr Leu Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser
35 40 45

Leu Arg Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn
50 55 60

Ile Leu Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu
65 70 75 80

Asn Ser Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile
85 90 95

Ser Pro Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln
100 105 110

Trp Arg His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys
115 120 125

Arg Gln Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val
130 135 140

Thr Trp Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp
145 150 155 160

Ile Tyr Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp
165 170 175

Thr Gly Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr
180 185 190

Glu Glu Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn
195 200 205

Gly Thr Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu
210 215 220

Ile Glu Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr
225 230 235 240

Val Arg Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys
245 250 255

Phe Phe Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr
260 265 270

Ser Ile Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr
275 280 285

Leu Cys Asp Val Thr Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp
290 295 300

Leu Arg Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp
305 310 315 320

Glu Ser Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu
325 330 335

Met Ser Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His
340 345 350

Phe Thr Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu
355 360 365

Gly Tyr Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr
370 375 380

Phe Ile Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr
385 390 395 400

Ser Asp Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly
405 410 415

Gly Arg Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr
420 425 430

Cys Leu Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val
435 440 445

Ser Phe Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro
450 455 460

Gly Leu Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu
465 470 475 480

Arg Val Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val
485 490 495

Gln Met Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys
500 505 510

Phe Trp Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys
515 520 525

Tyr Pro Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala
530 535 540

Asp Thr Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu
545 550 555 560

Asn Ile Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly
565 570 575

```
Asp Lys Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val
            580                 585                 590

Glu Asp Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val
        595                 600                 605

Asp Asn Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val
    610                 615                 620

Thr Ser Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile
625                 630                 635                 640

Ala Val Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr
                645                 650                 655

Glu Arg Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr
            660                 665                 670

Arg Asn Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu
        675                 680                 685

Tyr Leu Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln
    690                 695                 700

Ser Ala Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln
705                 710                 715                 720

Ala Met Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala
                725                 730                 735

His Gln His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe
            740                 745                 750

Ser Leu Pro
        755
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Lys Gly Leu Leu Gly
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGCCGACGA TGAAGGGACT GCTGGGTGCT 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Ile Thr Val Ala Thr Ala Asp Ser Arg
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala
1 5 10 15

Thr Ala Asp Ser Arg
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACCATCATCA CCGTGGCTAC AGCTGACAGT 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	double
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTACGTATCA TTAGCTATTC GA 22

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	593
(B) TYPE:	amino acid
(C) STRANDEDNESS:	
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
 1               5                  10                  15

Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr
            20                  25                  30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
        35                  40                  45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
    50                  55                  60

Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn
65                  70                  75                  80

Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp
                85                  90                  95

Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln
            100                 105                 110

Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr
        115                 120                 125

Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr
    130                 135                 140

Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val
145                 150                 155                 160
```

Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile
165 170 175

Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp
180 185 190

Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe
195 200 205

Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala
210 215 220

Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe
225 230 235 240

Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr
245 250 255

Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn
260 265 270

Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr
275 280 285

Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr
290 295 300

Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln
305 310 315 320

Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg
325 330 335

Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly
340 345 350

Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly
355 360 365

Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile
370 375 380

Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly
385 390 395 400

Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr
405 410 415

Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr
420 425 430

Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu
435 440 445

Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu
450 455 460

Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr
465 470 475 480

Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp
485 490 495

```
Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys
            500                 505                 510

Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met
        515                 520                 525

Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu
    530                 535                 540

Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg
545                 550                 555                 560

Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala
                565                 570                 575

Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His
            580                 585                 590

Ala
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Gly Asp Lys Ile Met His Ala
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Thr Pro Trp Lys Val Leu Leu
 1               5
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Trp Ser Tyr Gly
1 5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Gly Xaa Ser Xaa Gly
1 5

Other embodiments are within the following claims.

What is claimed is:

CLAIMS:

1. A nucleic acid encoding a polypeptide fragment of CD26 lacking amino acids 3-9 of intact CD26.

2. A nucleic acid encoding a polypeptide fragment of CD26 lacking amino acids 24-34 of intact CD26.

3. The nucleic acid of claim 1, wherein said polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2.

4. The nucleic acid of claim 2, wherein said polypeptide has an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 3.

5. A plasmid comprising the nucleic acid of any of claims 1 or 2.

6. A polypeptide fragment of CD26 capable of disrupting the naturally occurring binding interaction between CD45 and CD26.

7. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of CD45 present in said first immunoprecipitate is less than the amount of CD45 present in said second immunoprecipitate, the presence of a lesser amount of CD45 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

8. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of CD26 present in said first immunoprecipitate is less than the amount of CD26 present in said second immunoprecipitate, the presence of a lesser amount of CD26 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

9. A monoclonal antibody which, when contacted under physiological conditions with a cell expressing CD26 and CD45, interferes with the association of said CD26 and CD45.

10. A cell transfected with a nucleic acid encoding CD26, said cell expressing both CD26 and CD45 on its surface.

11. A cell transfected with a nucleic acid encoding CD45, said cell expressing both CD26 and CD45 on its surface.

12. A method of generating a hybridoma cell, said method comprising:

(a) providing a cell transfected with nucleic acid encoding CD26, such that said cell expresses CD26 on its surface;

(b) using said cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from said subject animal with a cell from an immortal cell line to produce a hybridoma cell.

13. A polypeptide fragment of CD26 capable of disrupting the naturally-occurring binding interaction between p43 and CD26.

14. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to p43, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and p43;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of p43 present in said first immunoprecipitate is less than the amount of p43 present in said second immunoprecipitate, the presence of a lesser amount of p43 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

15. A polypeptide comprising the amino acid sequence of CD26 carboxy-terminal to Ala37, wherein at least one of the amino acids in the segment Gly627-Gly631 is deleted or replaced with a different amino acid.

16. A polypeptide fragment of CD26 lacking residues 1-34 of intact CD26.

17. A vaccine adjuvant comprising a fragment of CD26 in a pharmaceutically acceptable carrier.

18. A method of screening candidate immunosuppressive compounds, said method comprising:

(a) contacting a lymphocyte with CD26 or a fragment of CD26 in the presence of a candidate compound, and

(b) determining whether said candidate compound inhibits the CD26-enhanced proliferation of said lymphocyte, said inhibition being an indication that said candidate compound has immunosuppressive activity.

19. CD26 or a fragment thereof affixed to a solid matrix material.

FIG. 1

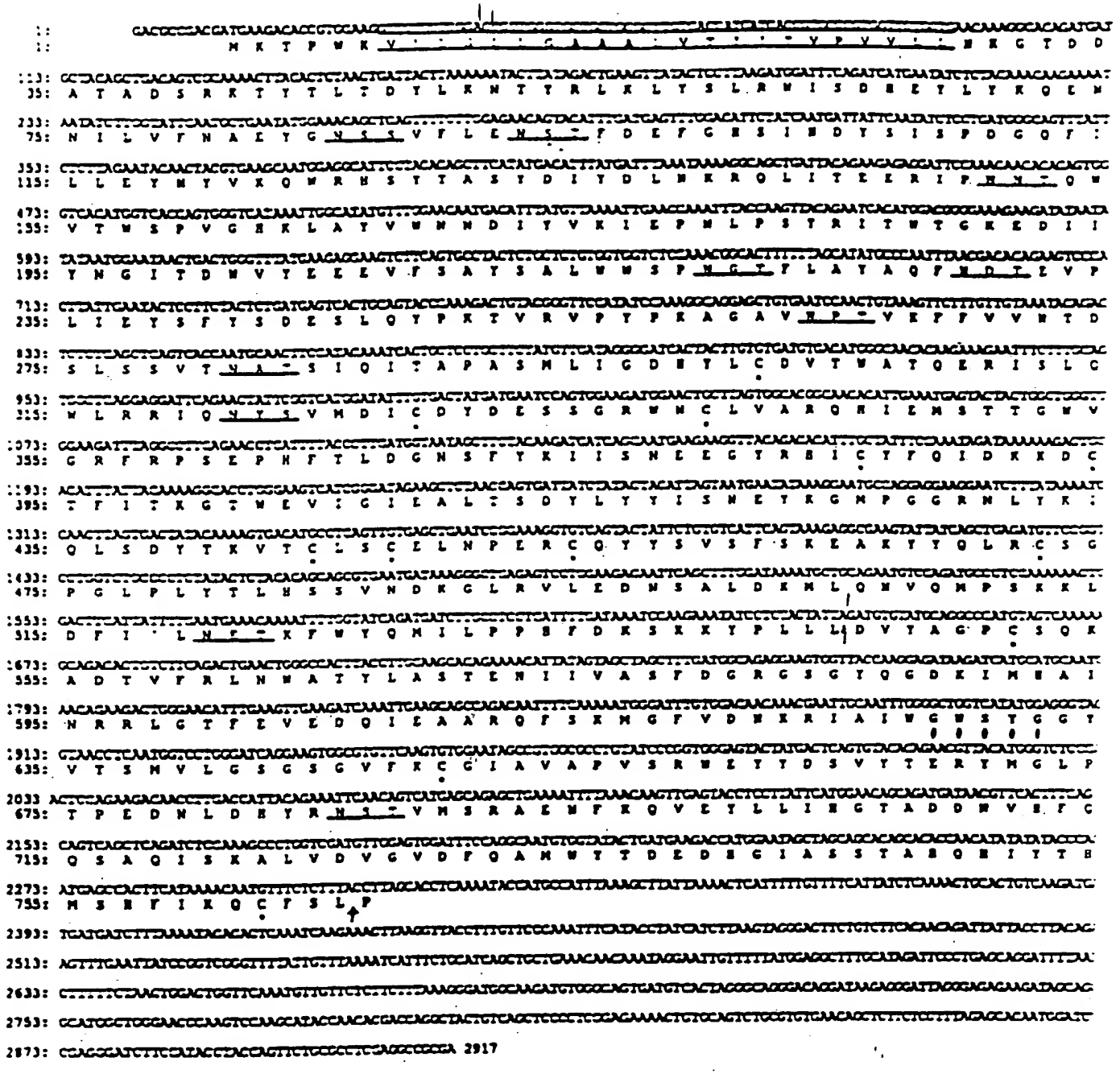


FIG. 2

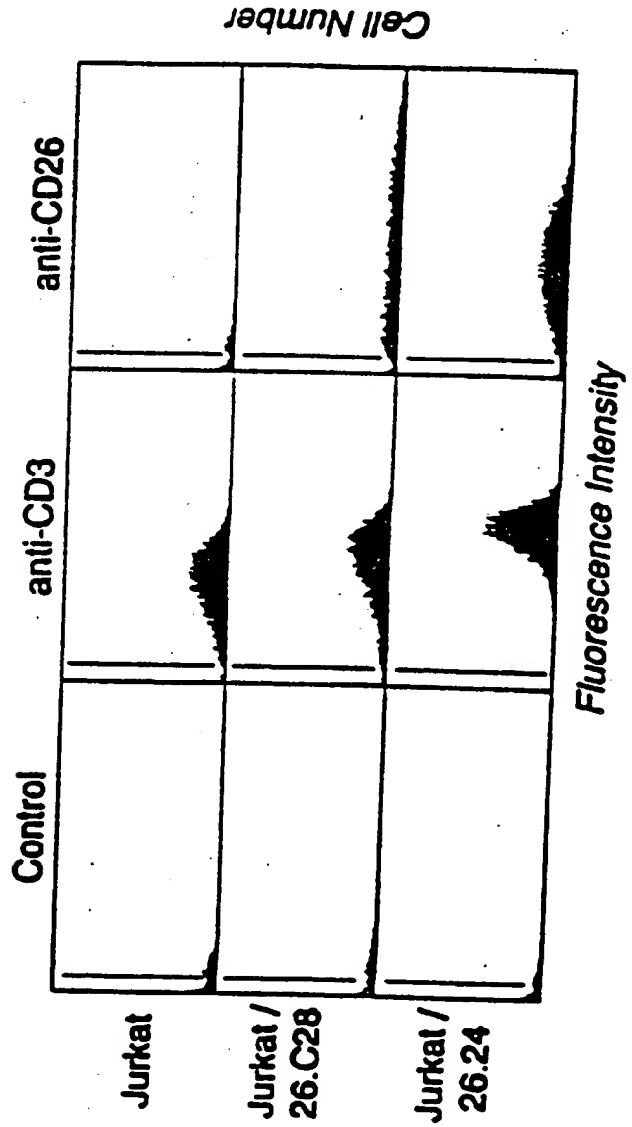

Cell Number
anti-CD26
anti-CD3
Control
Fluorescence Intensity
Jurkat
Jurkat / 26.C28
Jurkat / 26.24

FIG. 3A

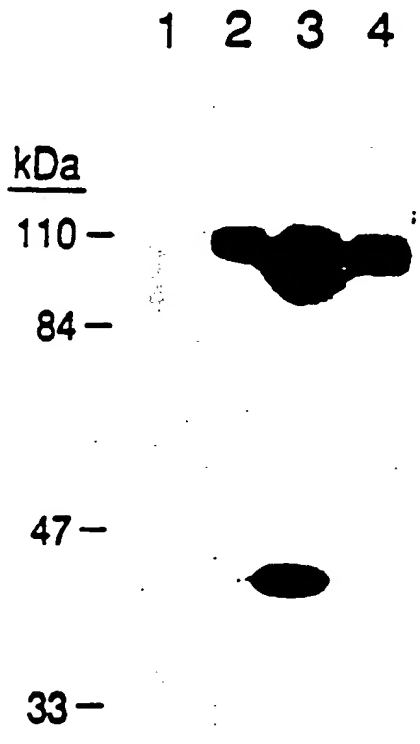
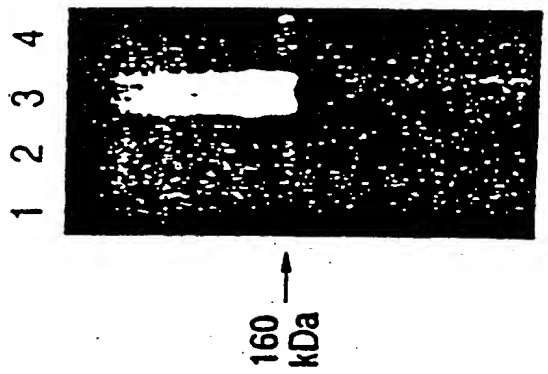


FIG. 3B



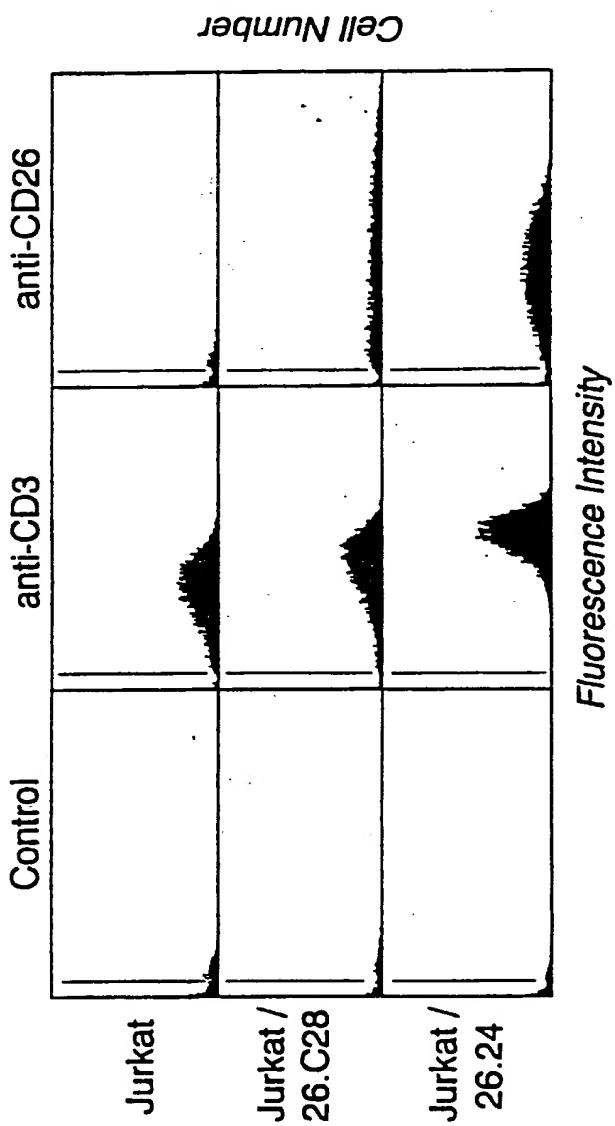

Cell Number
anti-CD26
anti-CD3
Control
Fluorescence Intensity
Jurkat
Jurkat / 26.C28
Jurkat / 26.24

FIG. 2

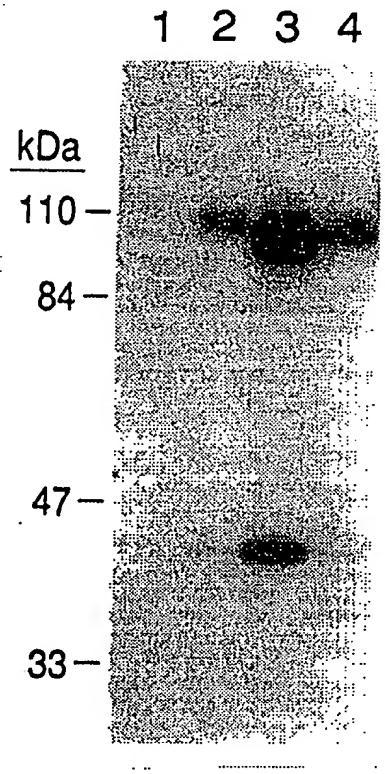


FIG. 3A

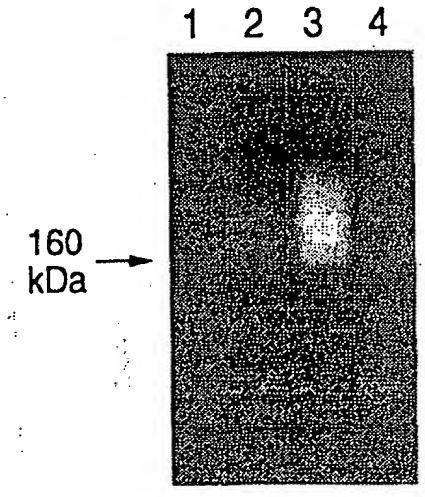


FIG. 3B

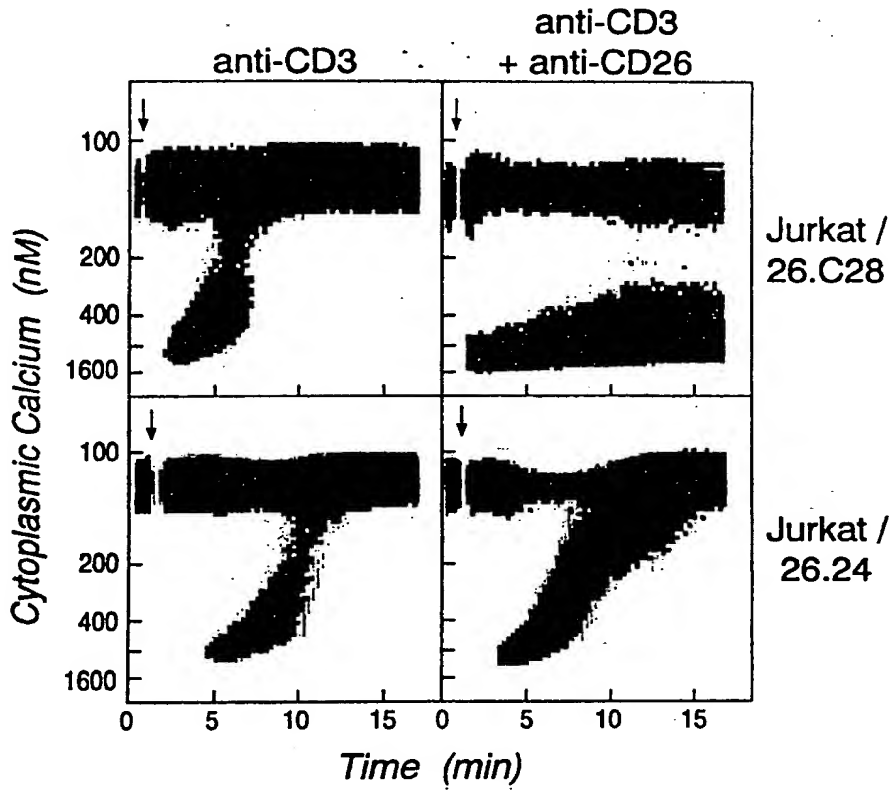

anti-CD3
anti-CD3
+ anti-CD26
Cytoplasmic Calcium (nM)
100
200
400
1600
Jurkat /
26.C28
Jurkat /
26.24
0
5
10
15
Time (min)

FIG. 4

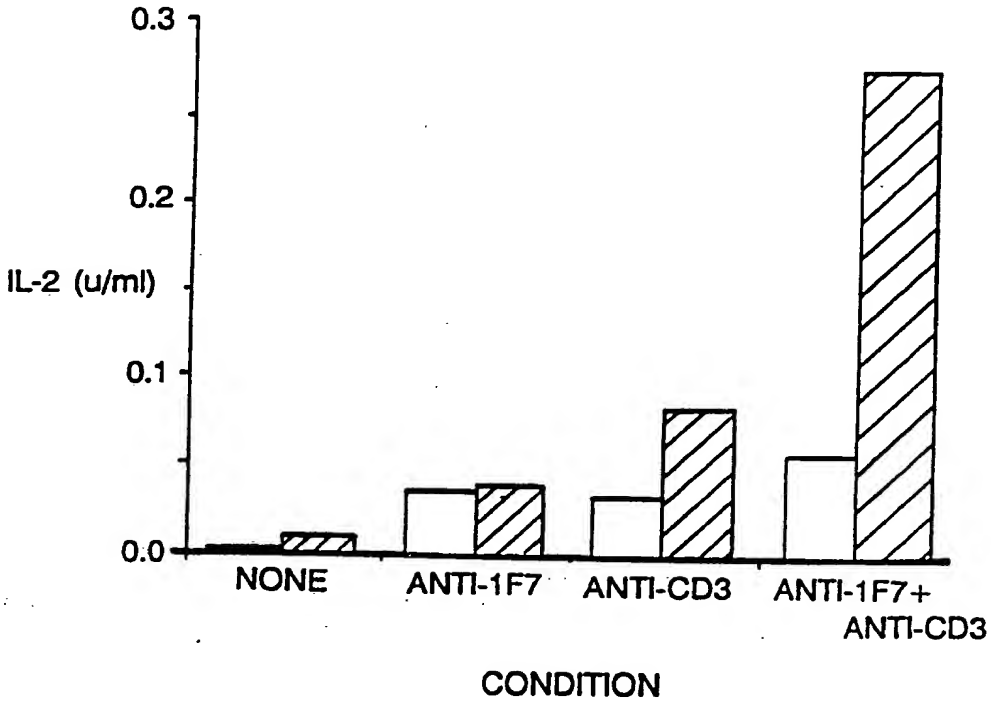

0.3
0.2
IL-2 (u/ml)
0.1
0.0
NONE
ANTI-1F7
ANTI-CD3
ANTI-1F7+
ANTI-CD3
CONDITION

FIG. 5

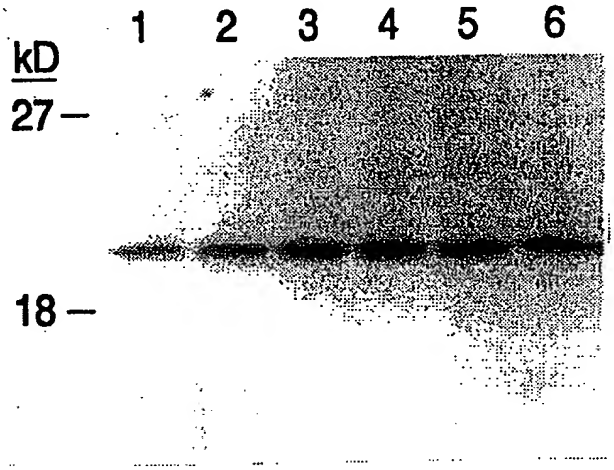


FIG. 6

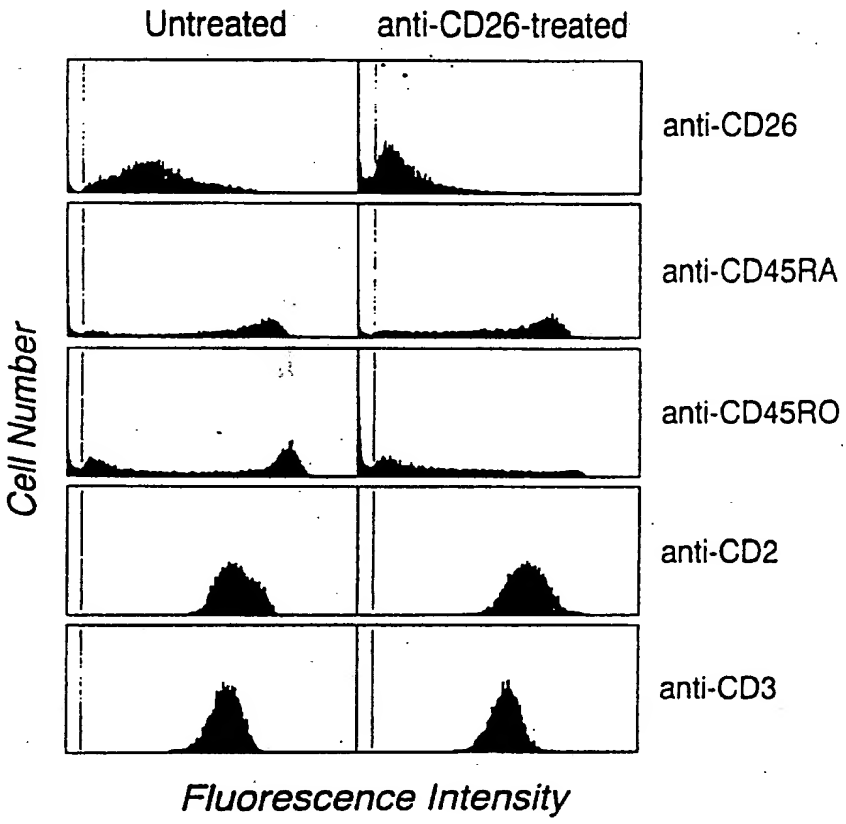

Untreated
anti-CD26-treated
anti-CD26
anti-CD45RA
anti-CD45RO
anti-CD2
anti-CD3
Cell Number
Fluorescence Intensity

FIG. 7

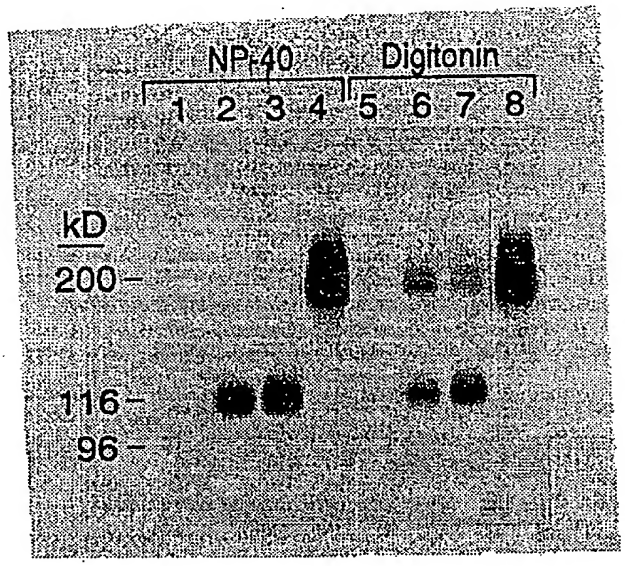


FIG. 8

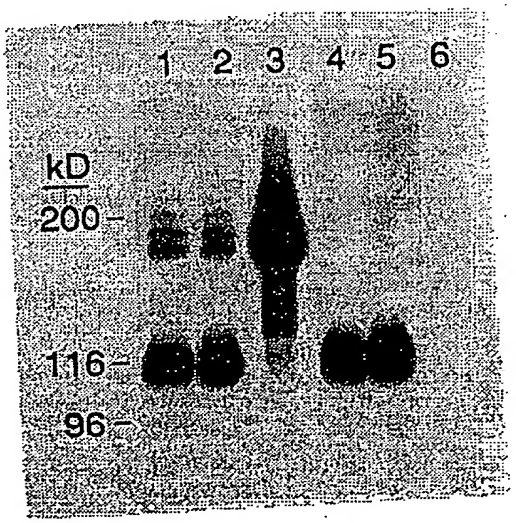


FIG. 9

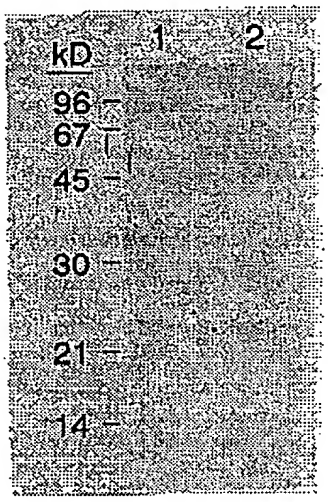


FIG. 10

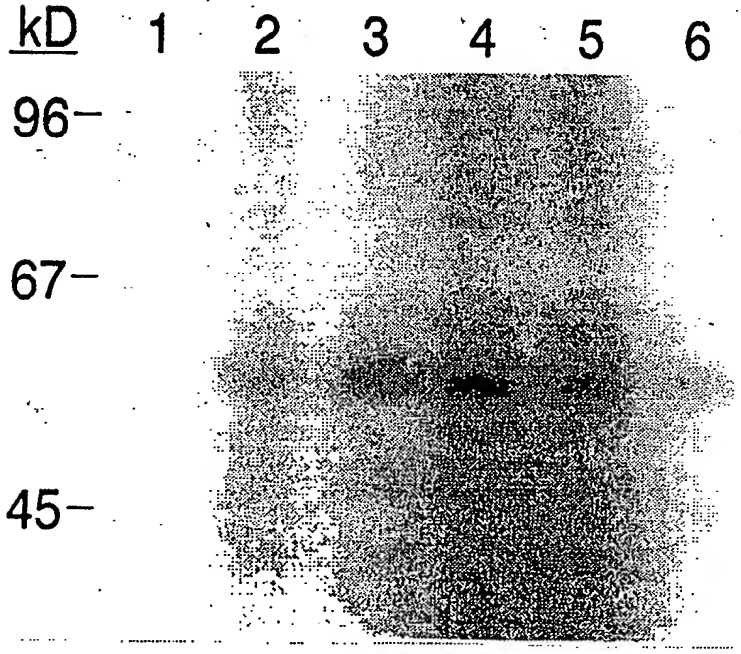


FIG. 11

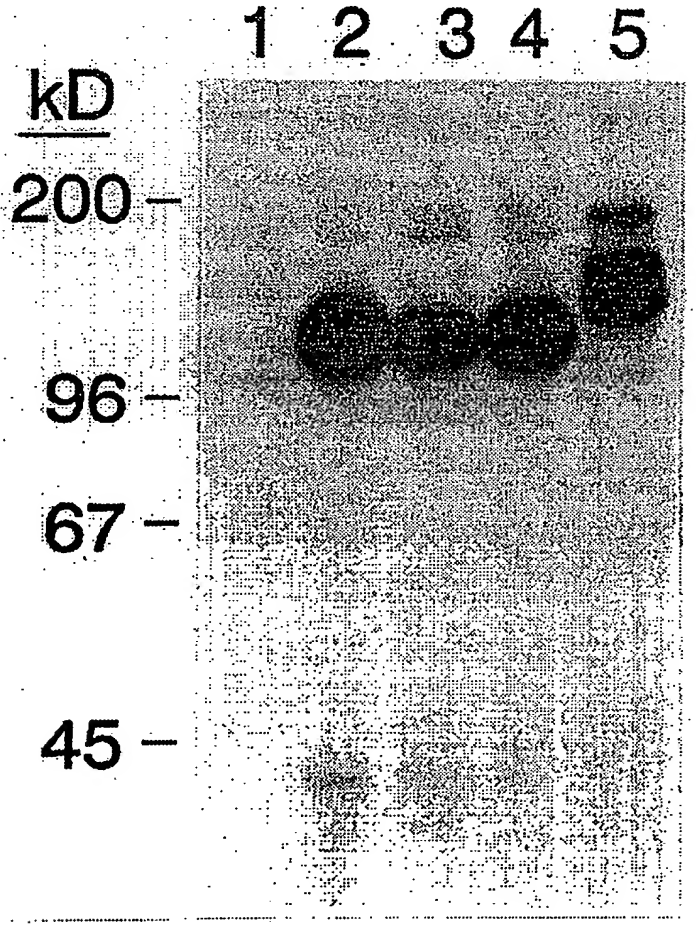


FIG. 12

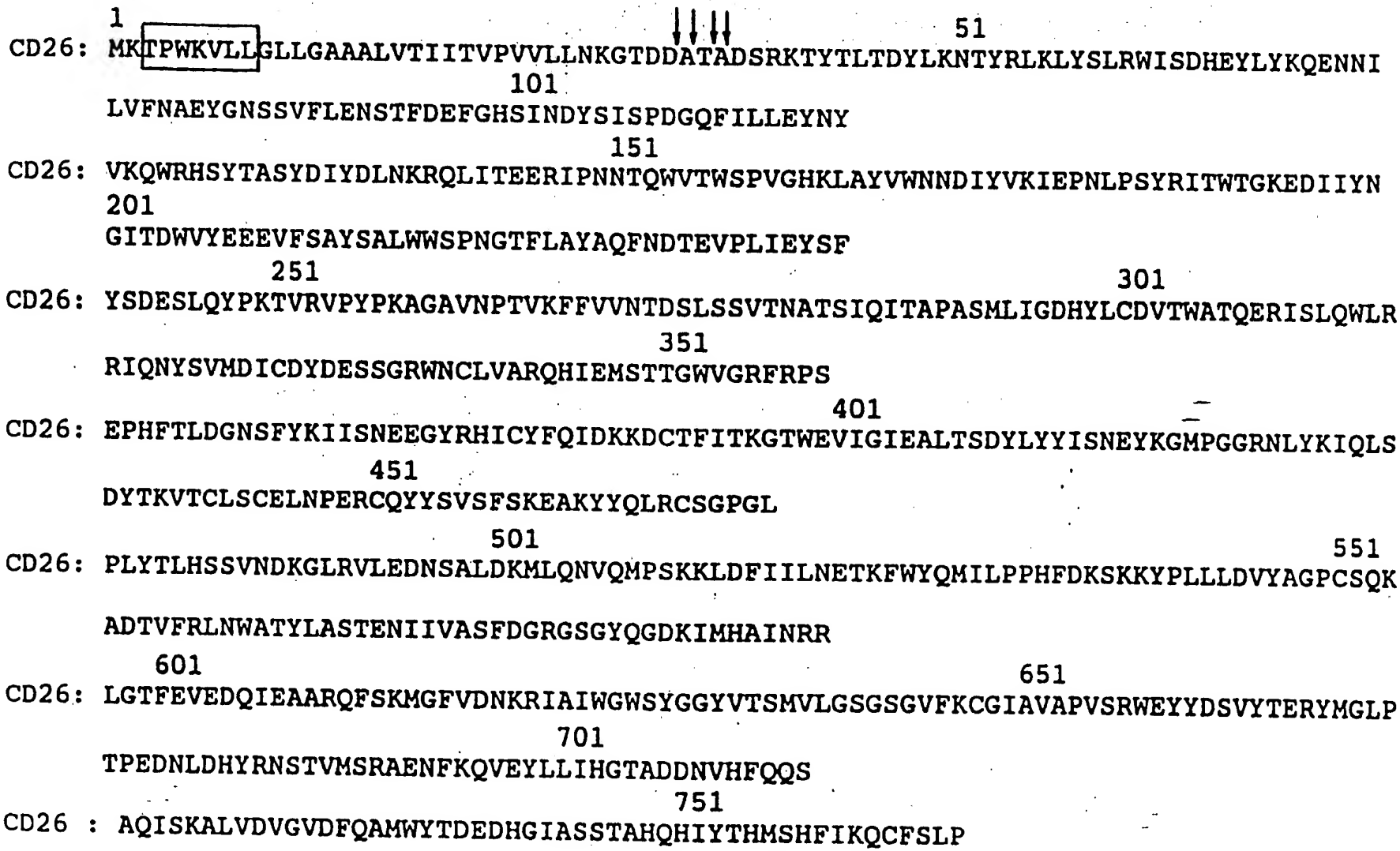


FIG. 13

FIG. 14

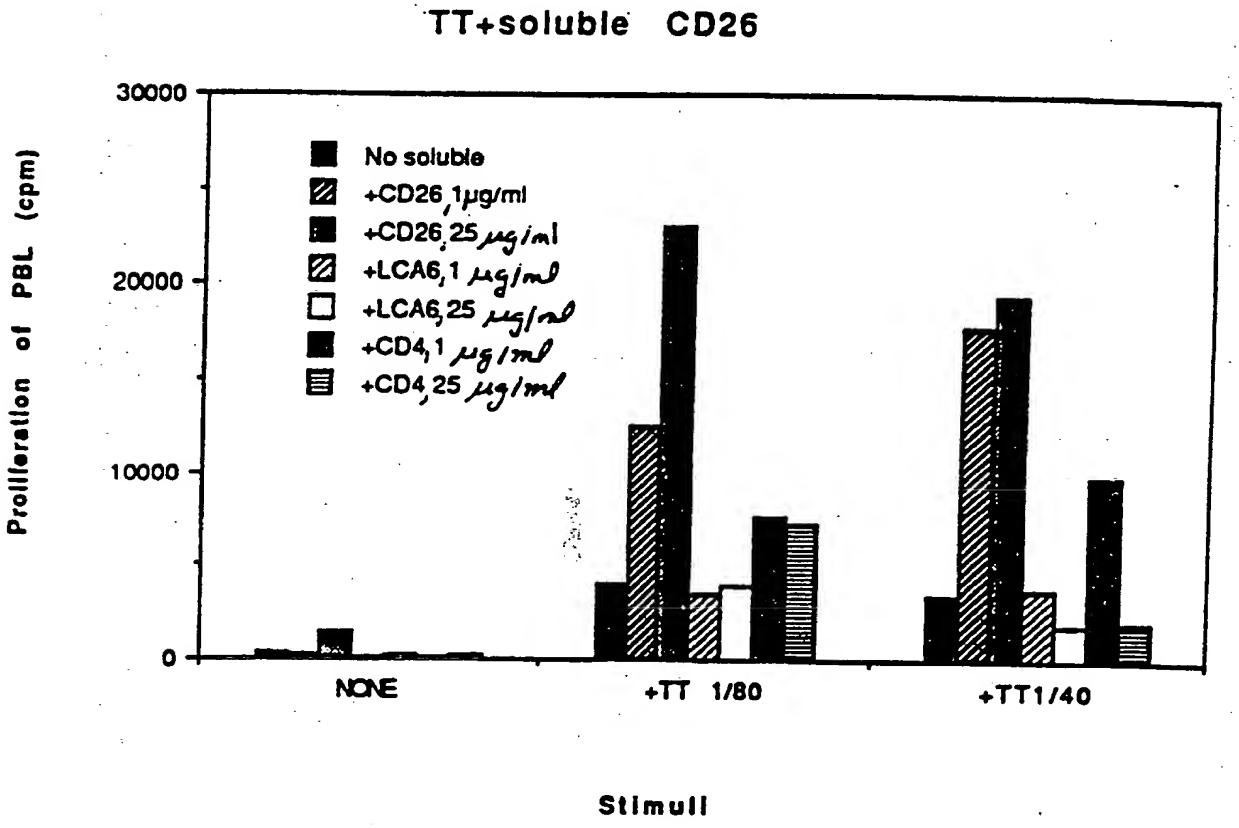
```
          1                                                  51
CD26: MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSL

                                                  101
      RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNY
                                        151
      VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL
                               201
      PSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSF
                    251
      YSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYL
      301                                                351
      CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS

                                                  401
      EPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN
                                        451
      EYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY
                               501
      TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKY
                    551
      PLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHA
```

FIG. 15

FIG. 16


TT+soluble CD26
30000
20000
10000
0
Proliferation of PBL (cpm)
No soluble
+CD26,1µg/ml
+CD26,25µg/ml
+LCA6,1 µg/ml
+LCA6,25 µg/ml
+CD4,1 µg/ml
+CD4,25 µg/ml
NONE
+TT 1/80
+TT1/40
Stimuli

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07923

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :Please See Extra Sheet.
US CL :Pleas See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 435/320.1, 240.2, 240.27, 69.1, 7.24; 530/395, 388.7, 402; 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Volume 264, Number 6, issued 25 February 1989, S. Ogata *et al.*, "Primary Structure of Rat Liver Dipeptidyl Peptidase IV Deduced from Its cDNA and Identification of the NH_2-terminal Signal Sequence as the Membrane-anchoring Domain", pages 3596-3601, especially the abstract.	1-19
Y	Biochimica et Biophysica Acta, Volume 1131, issued 1992, Y. Misumi *et al.*, "Molecular cloning and sequence analysis of human dipeptidyl peptidase IV, a serine proteinase on the cell surface", pages 333-336, especially the abstract.	1-8, 11-19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the internati nal search
05 November 1993

Date f mailing f the international search report
NOV 18 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07923

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Biological Chemistry, Volume 263, Number 32, issued 15 November 1988, S. R. Schmid *et al.*, "Deletion of the Amino-terminal Domain of Asialoglycoprotein Receptor H1 Allows Cleavage of the Internal Signal Sequence", pages 16886-16891, especially the abstract.	15-19
Y	Proceedings of the National Academy of Sciences of the USA, Volume 84, issued December 1987, A. Aruffo *et al.*, "Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system", pages 8573-8577, especially the abstract.	1-19
Y	Methods in Enzymology, Volume 152, issued 1987, W. I. Wood, "Gene Cloning Based on Long Oligonucleotide Probes", pages 443-447, see the entire document.	1-19
A	Molecular Immunology, Volume 29, Number 2, issued February 1992, Y. Torimoto *et al.*, "Biochemical characterization of CD26 (dipeptidyl peptidase IV): functional comparison of distinct epitopes recognized by various anti-CD26 monoclonal antibodies", pages 183-192.	1-19
A	Biochemistry, Volume 28, Number 21, issued 1989, W. Hong *et al.*, "Expression of Enzymatically Active Rat Dipeptidyl Peptidase IV in Chinese Hamster Ovary Cells after Transfection", pages 8474-8479.	1-19
A	Scandanavian Journal of Immunology, Volume 31, Number 4, issued April 1990, A. J. Ulmer *et al.*, "CD26 Antigen is a Surface Dipeptidyl Peptidase IV (DPPIV) as Characterized by Monoclonal Antibodies Clone TII-19-4-7 and 4EL1C7", pages 429-435.	1-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07923

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation f document, with indication, where appropriate, f the relevant passages	Relevant to claim N .
Y	Journal of Biological Chemistry, Volume 267, Number 7, issued 05 March 1992, D. Darmoul *et al.*, "Dipeptidyl Peptidase IV (CD 26) Gene Expression in Enterocyte-like Colon Cancer Cell Lines HT-29 and Caco-2: cloning of the complete human coding sequence and changes of dipeptidyl peptidase IV mRNA levels during cell differentiation", pages 4824-4833, especially the abstract.	1-19
Y	Journal of Immunology, Volume 147, Number 8, issued 15 October 1991, Y. Torimoto *et al.*, "Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes", pages 2514-2517, especially the abstract and page 2517.	7-11
X	Journal of Immunology, Volume 141, Number 11, issued 01 December 1988, M. Streuli *et al.*, "Characterization of CD45 and CD45R monoclonal antibodies using transfected mouse cell lines that express individual human leukocyte common antigens", pages 3910-3913, especially the abstract.	9
X	Journal of Immunology, Volume 149, Number 4, issued 15 August 1992, G. A. Koretzky *et al.*, "Restoration of T cell receptor-mediated signal transduction by transfection of CD45 cDNA into a CD45-deficient variant of the Jurkat T cell line", pages 1138-1142, especially the abstract.	11
Y	Journal of Cell Biology, Volume 111, issued August 1990, W. Hong *et al.*, "Molecular Dissection of the NH_2-Terminal Signal/Anchor Sequence of Rat Dipeptidyl Peptidase IV", pages 323-328, especially the abstract.	13, 15-19
Y,P	Science, Volume 261, issued 23 July 1993, J. Kameoka *et al.*, "Direct Association of Adenosine Deaminase with a T Cell Activation Antigen, CD26", pages 466-469, especially the abstract.	13, 14
Y	Nature, Volume 334, issued 11 August 1988, S. Brenner, "The molecular evolution of genes and proteins: a tale of two serines", pages 528-530, especially the abstract and Table I.	15
Y	Journal of Biological Chemistry, Volume 266, Number 15, issued 25 May 1991, G. Pei *et al.*, "Expression, Isolation, and Characterization of an Active Site (Serine-528-->Alanine) Mutant of Recombinant Bovine Prothrombin", pages 9598-9604, especially the abstract.	15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07923

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

C12N 15/12, 15/10, 15/85, 5/10, 5/12; C07K 13/00, 17/02, 15/28; A61K 37/02

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.2; 435/320.1, 240.2, 240.27, 69.1, 7.24; 530/395, 388.7, 402; 514/2, 12

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Text databases: Medline, Biosis, SciSearch, CAS, Embase, USPTO-APS
Search terms: CD26, thymocyte activating molecule, dipeptidyl peptidase IV; CD45, leukocyte common antigen; clon?, recombinant, cDNA, mRNA, PCR, transfect?
Sequence databases: GenBank, EMBL, GeneSeq, SwissProt, PIR